PHOTOSYNTHETICA 34 (3): 447-464, 1997

Flow cytometric characteristics of subchloroplast particles prepared by the action of various detergents

N. WILHELMOVÁ*, S. VANĚK** and J. WILHELM***

*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, CZ-160 00 Praha 6, Czech Republic**
*Department of Clinical Haematology of the Faculty Hospital of Charles University, U nemocnice 1, CZ-128 21 Praha 2, Czech Republic***
*2nd Medical School, Charles University, Plzeňská 221, CZ-150 00 Praha 5, Czech Republic****

Abstract

Agranal thylakoid membranes from leaves of *Phaseolus vulgaris* L. were fragmented using seven distinct detergents: digitonin, *Triton X-100*, cetylpyridinium chloride, sodium dodecylsulfate, and *Zwittergents* 3-12, 3-14, and 3-16, differing in chemical composition and/or electric charges. Subchloroplast particles separated on a *Percoll* gradient were examined by flow cytometry to determine their size and shape. Vesicle size was also determined by a haematological analyzer, which produced comparable results. Individual green bands consisted of vesicles of fairly wide size distribution. Simple direct proportionality between the particle density and their size was not observed in any case, nevertheless, bigger particles were more abundant in fractions of higher density. Some vesicles had even a larger size than the original thylakoids. This might reflect a specific action of the detergents in low concentrations on agranal membranes, with incorporation of detergent molecules into vesicles. Inner structures of particles of the same size and density were not necessarily identical, but represented several populations, as was apparent from the side scatter analysis. Flow cytometric analysis can thus be used for the investigation of mechanisms of membrane fragmentation by detergents.

Additional key words: cetylpyridinium chloride; digitonin; membrane vesicles; *Phaseolus vulgaris;* sodium dodecylsulfate; thylakoids; *Triton X-100*; *Zwittergents*.

Introduction

Detergents are widely used for preparation of chloroplast fragments which serve as a starting material for investigation of the functional organization of photochemical

Received 4 July 1996, *accepted* 9 January 1997.
*Fax: (+420-2) - 243 10 109; e-mail: sestak@site.cas.cz
Acknowledgement: This work was financially supported by the Grant Agency of the Czech Republic (Project No. 206/95/1302).

processes. Though the composition of these preparations is known in great detail (*e.g.*, Antonopoulou and Akoyunoglou 1986, Ort 1986, Camm *et al.* 1987, Herrin *et al.* 1987, Hansson and Wydrzinski 1990), they are less well characterized with respect to their size and particle size distribution.

In order to investigate this problem, we chose seven different detergents and observed their effects on chloroplast fragmentation. We measured the size of the particles by two independent methods (conductivity and light scatter), determined their size distribution and structural characteristics, and also chlorophyll fluorescence in dependence on particle size by flow cytometry. We have chosen the minimal detergent concentration that was able to fragment thylakoid membranes. As a starting material, low-salt agranal thylakoid membranes were used, so that we could observe the effect of detergents on single, unstacked membranes. For separation of fragments we used centrifugation in isoosmotic *Percoll* gradient which enabled separation of osmotically undisturbed particles and comparison of particles' real size and buyoant density.

Materials and methods

French bean (*Phaseolus vulgaris* L. cv. Jantar) plants were grown in sand cultures in the growth chamber (*Kovodružstvo*, Slaný, Czech Republic) under constant temperature of 25/16 °C, air humidity 60/80 %, and fluorescence tubes irradiance 300/0 μmol m^{-2} s^{-1} PAR (day/night). The duration of photoperiod was 16 h. Plants were twice a week supplied with an IBP nutrition solution, on the other days with distilled water (Šesták *et al.* 1978). Primary leaves were used for analysis, buds of other leaves were continuously removed.

Digitonin and sodium dodecylsulfate (SDS) were purchased from *Sigma*, *Triton X-100*, and cetylpyridinium chloride (CPC) from *Koch-Light*, *Zwittergents* 3-12, 3-14, and 3-16 from *Calbiochem*. *Percoll* and density marker beads from *Pharmacia*. Class II chloroplasts were prepared from 18 d-old bean leaves according to Jursinic and Govindjee (1977). They were twice washed and resuspended in the same buffer, 0.05 M Tricine, pH 7.3. The result of this procedure were agranal thylakoid membranes. These membranes were incubated with different detergents for 30 min at 20 °C in the dark in a thermostated shaker. An amount of membrane suspension equivalent to 0.525 mg of chlorophyll was mixed with the particular detergent to make a total volume of 0.75 cm^3. The following final concentrations of individual detergents were used: digitonin and CPC 0.3 mM, all other detergents 0.03 mM. These detergent concentrations represent the minimal necessary amount of detergent causing membrane fragmentation in our conditions (Wilhelmová 1994). Detergent-disrupted membranes were applied on the top of a centrifugation tube filled with a *Percoll* solution (35 % *Percoll* in 0.25 M sucrose, pH 7.3) that contained the same concentration of a particular detergent. The tubes were spun for 1 h at 50 000 × *g* on a *Beckman J2-21M/E* centrifuge. The shape of density gradient was checked with the aid of density marker beads which were spun in a separate tube. Green zones of

thylakoid fragments were separated with flat-tipped syringe needle, and their chlorophyll content was determined according to Arnon (1949).

The measurements of particles' characteristics were performed on a flow cytometer *Facscan* (*Becton Dickinson*) which enabled parallel analysis of three parameters on each event. These were: forward scatter of laser beam which corresponded to the size of a particle, side (right angle) scatter indicating granularity and/or structural features of a particle, and red fluorescence which was detected for wavelengths longer than 650 nm. All parameters were measured as relative values with respect to the setting of the instrument.

The instrument was equipped with argon-ion laser as a source of radiation at 488 nm. The "list mode" of measurement was used (*i.e.*, all parameters were measured and stored in computer memory for analysis) with the aid of *Facscan* Research Software run on the *Consort 30* computer (*Hewlet Packard*). Each measured parameter was printed as a histogram (forward scatter, side scatter, and red fluorescence), and correlations between parameters were illustrated with the help of dot plot diagrams (forward scatter *versus* side scatter, and forward scatter *versus* red fluorescence). Adjustment of the instrument was set by blank microbeads with diameters 4.9, 5.8, and 9.0 μm, and by beads with a diameter 7.3 μm labelled with chlorophyll (*Flow Cytometry Standards Corporation*).

The size of thylakoid membrane particles was also measured on a haematological analyzer *Coulter Counter S Plus STKR*, in which the measurement was based on conductivity changes caused by the particles. The measurements were run in the "red blood cells channel", *i.e.*, without osmotic lysis of the particles. The thylakoid membrane particles fit by their size to a region occupied in blood by platelets. The diameter of the particles was computed from their volumes corresponding to the peaks in histogram presentation.

For a comparison of both methods, microbeads of defined size were measured on the haematological analyzer. The results were as follows (the measured value is in parentheses): 4.9 μm (4.2 μm), 5.8 μm (5.6 μm), 7.3 μm (7.5 μm), 9.0 μm (8.1 μm). A qualitative agreement between the values is apparent, the absolutely lower values produced by the haematological analyzer might be caused by different conductivities of the measured particles.

Results

As a starting material low-salt agranal thylakoid membranes were used, so that we could observe the effects of detergents on single, unstacked membranes. We choose the minimal detergent concentration that was able to fragment thylakoid membranes. The thylakoid fragments obtained after detergent treatment were separated by centrifugation in the isoosmotic gradients of *Percoll* (Fig. 1) which, to the contrary of sucrose gradients, enabled separation of osmotically undisturbed particles, and thus a comparison of particles' size and buyoant density. The *Percoll* layers used as controls were labeled as P1 to P3. Fig. 2 illustrates the shape of the *Percoll* density gradient in the centrifugation tube after centrifugation at 50 000 × *g* for 1 h. After separation, the

particle volumes in the individual fractions was determined with the haematological analyzer *Coulter Counter S Plus STKR*. The values summarized in Table 1 represent

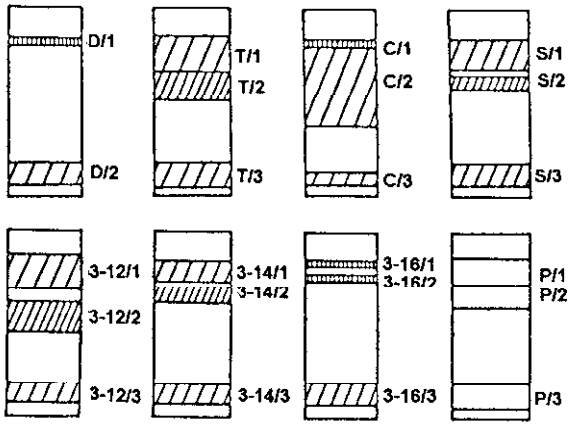

Fig. 1. Fractionation pattern of fragments obtained after action of various detergents. D = 0.3 mM digitonin, T = 0.03 mM *Triton X-100*, C = 0.03 mM cetyl-pyridinium chloride, S = 0.03 mM SDS, 3-12 (14,16) = 0.03 mM *Zwittergent* 3-12 (14,16). Tubes were spun at 50 000 × *g* for 1 h.

the volumes corresponding to the peaks in histogram of size distribution. An estimate of particles' diameter was based on an assumption that the particles were ball-shaped. This assumption was substantiated by inspecting the particles under a fluorescence microscope.

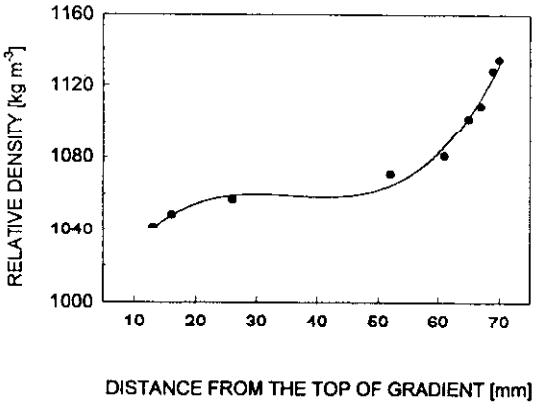

Fig. 2. The shape of 35 % *Percoll* gradient after centrifugation at 50 000 × *g* for 1 h.

The particles were further investigated by flow cytometry (Figs. 5-11). As the measurements were run in the presence of *Percoll*, we determined the characteristics of *Percoll* fractions of different density (P1, P2, P3, see Fig. 1). The forward scatter (which corresponded to the size of particles) laid in the range of 7 to 100 relative units and denser particles were shifted to higher values (Fig. 3). By comparison with the results presented in Table 1, this range corresponded to 1.6-2.8 μm of diameter. A characteristic feature of *Percoll* particles was their low side scatter (less than 10 relative units) caused by the nature of the particles.

Table 1. Characteristics of membrane vesicles from individual gradient fractions measured by haematological analyzer. [a] - values represent the peaks of the histogram, [b] - calculated values.

Detergent	Fraction	Particle amount [$\times 10^6$ m^{-3}]	density [$\times 10^3$ kg m^{-3}]	volume[a] [$\times 10^{-12}$m^3]	diameter[b] [μm]
digitonin	D/1	7	1.047	3; 7	1.8; 2.4
	D/2	2	1.110	3; 6; 9	1.8; 2.4; 2.6
Triton X-100	T/1	11	1.052	2	1.6
	T/2	10	1.058	2; 5	1.6; 2.1
	T/3	4	1.118	3; 14; 17; 40; 45	1.8; 3.0; 3.2; 4.2; 4.4
CPC	C/1	11	1.044	3; 7	1.8; 2.4
	C/2	7	1.057	3; 5; 6	1.8; 2.1; 2.4
	C/3	4	1.118	3; 7; 14; 19; 40; 48	1.8; 2.4; 3.0; 3.3; 4.2; 4.5
SDS	S/1	11	1.051	3; 6	1.8; 2.4
	S/2	12	1.056	3; 6	1.8; 2.4
	S/3	3	1.121	3; 12; 18; 30; 45	1.8; 2.8; 3.2; 3.9; 4.4
Zwittergent	3-12/1	9	1.051	3; 6	1.8; 2.4
3-12	3-12/2	9	1.060	2; 7	1.6; 2.4
	3-12/3	3	1.118	3; 5; 14; 18; 30; 50	1.8; 2.1; 3.0; 3.2; 3.9; 4.6
Zwittergent	3-14/1	2	1.050	3; 18; 30	1.8; 3.2; 3.9
3-14	3-14/2	14	1.054	3; 7	1.8; 2.4
	3-14/3	10	1.112	3; 6	1.8; 2.4
Zwittergent	3-16/1	21	1.044	3; 7; 30; 45	1.8; 2.4; 3.9; 4.4
3-16	3-16/2	18	1.051	3; 6; 30; 45; 55	1.8; 2.4; 3.9; 4.4; 4.7
	3-16/3	3	1.114	3	1.8
none	P1	1	1.049	2; 7; 9	1.6; 2.4; 2.6
(*Percoll*	P2	1	1.055	3; 5; 7; 11	1.8; 2.1; 2.4
particles)	P3	2	1.110	3; 8; 10	1.8; 2.4; 2.8

Fig. 4 shows the results of flow cytometric analysis of thylakoid membranes before treatment with detergents. Dot plot analysis of forward scatter *versus* side scatter (Fig. 4*A*) indicated that the majority of particles had a similar size as the *Percoll* particles (as apparent also from histogram 4*D*), but had much higher side scatter. This is illustrated in histogram 4*C*; the position of marker beads in this histogram is indicated by the peak around 1000 relative units. Dot plot analysis of forward scatter *versus* fluorescence intensity (4*B*) indicated linear increase of fluorescence intensity with increasing forward scatter. A similar increase was observed in side scatter with increasing forward scatter; these results can be tentatively explained as a formation of aggregates of smaller particles. Nevertheless, the histogram 4*D* showed two separate populations of particles, the bigger ones corresponded to the size of marker beads (4.9 and 7.3 μm). Although the *Percoll* particles did not fluoresce, in order to exclude the possible interference of scattered radiation in the fluorescence channel, fluorescence intensities lower than 80 relative units per particle were not taken into consideration, and thus the used range of

fluorescence intensities was 80 to 10 000 relative units per particle. The histogram of fluorescence intensities (4*E*) indicated the presence of two main populations of fluorescing particles: one had peak value around 300, the other around 2000. The peak around 1000 belonged to marker beads.

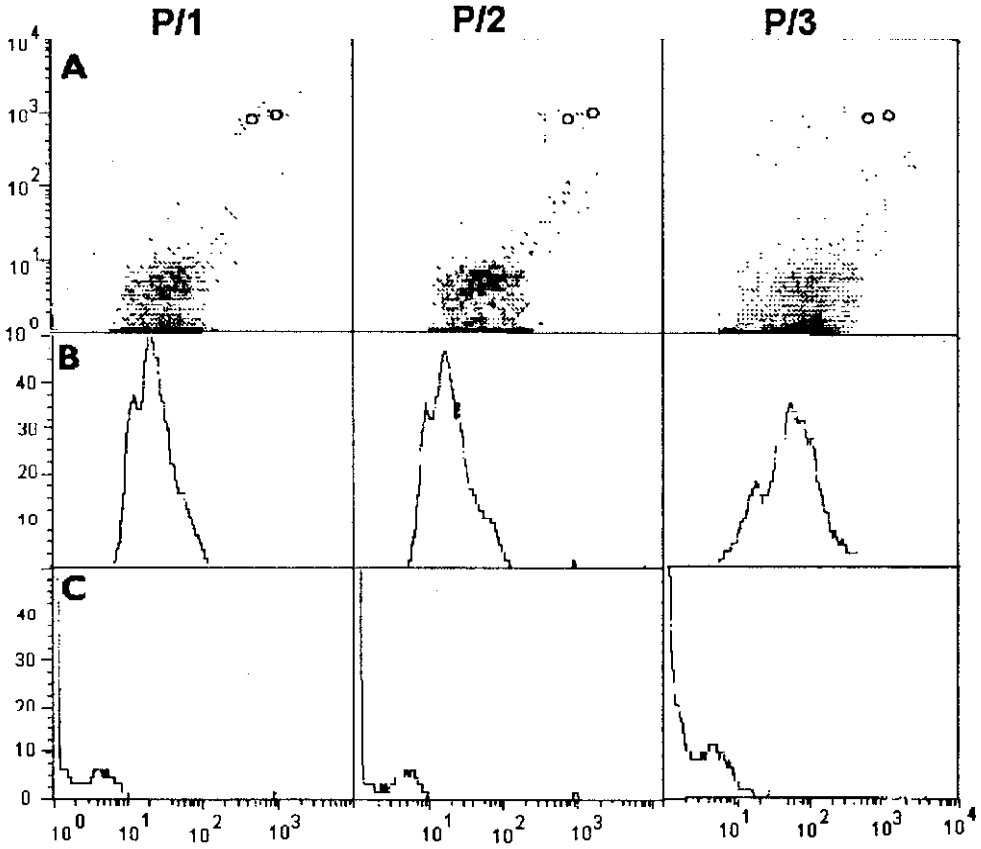

Fig. 3. Flow cytometric characteristics of three different layers (P/1 to P/3 in Fig. 1) of *Percoll* gradient. *A*: Dot plot analysis, *ordinate* = side scatter, *abscissa* = forward scatter. *B*: Histogram of forward scatter values. *C*: Histogram of side scatter values. The circles in *A* indicate the position of marker beads (4.9 and 7.3 μm). The scales are in relative units.

After digitonin treatment, two fractions were separated. The upper fraction D/1 with buyoant density 1.047 contained greater number of particles in unit volume (Table 1) than the lower fraction D/2 with buyoant density 1.110. Flow cytometric analysis of these fractions (Fig. 5) showed that fraction D/1 consisted of small particles of the diameter similar to that of the *Percoll* particles, an assessment in agreement with the values presented in Table 1, but they had a higher side scatter. The characteristic fluorescence intensity of this fraction was about 150 relative units per particle, but a small population of particles was characterized by fluorescence around 2000 relative units per particle (Fig. 5, D/1, *E*). The peaks around 1000

relative units in histograms *C*, *D*, *E* indicated the position of marker beads. The denser fraction D/2 differed markedly from the fraction D/1. First, it consisted of particles with much wider size distribution, and particles bigger than 7.3 μm were present. Some of the D/2 particles were even bigger than thylakoids (Fig. 4*D*). Second, the characteristic side scatter of these particles was around 300 compared to 15 of those of the D/1 fraction. And third, the majority of particles had fluorescence intensity around 2500 relative units per particle. A smaller population of particles had fluorescence intensity around 200, but these particles were much bigger than the corresponding particles of fraction D/1 with a similar fluorescence.

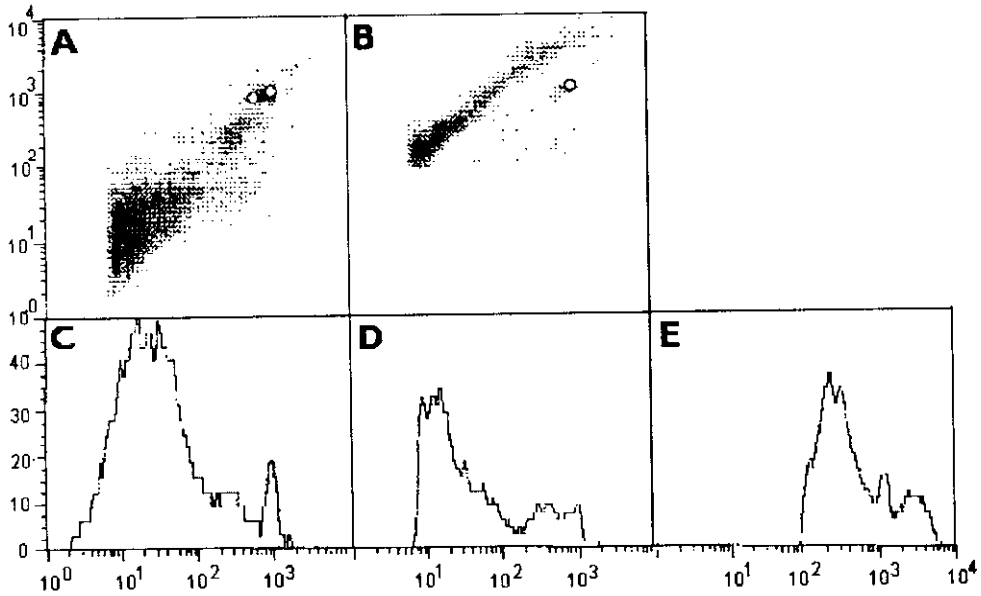

Fig. 4. Flow cytometric characteristics of thylakoid membranes before fractionation with detergents. *A*: Dot plot analysis, *ordinate* = side scatter, *abscissa* = forward scatter. *B*: Dot plot analysis, *ordinate* = fluorescence intensity, *abscissa* = forward scatter. *C*: Histogram of side scatter values. *D*: Histogram of forward scatter values. *E*: Histogram of fluorescence values. The circles in *A* and *B* indicate the positions of marker beads. The diameter of the beads was 1.9 and 7.3 μm in *A*, and 7.3 μm in B. *Abscissa* in histograms indicates the respective relative units in a logarithmic scale.

The *Triton X-100* treatment resulted in formation of three fractions of different buyoant densities (Table 1). The fraction T/3 contained less than half of the particles per unit volume than the upper fractions. The flow cytometric analysis (Fig. 6) showed that fractions T/1 and T/2 had almost identical properties. Therefore they could represent particles of the same size and structure differing in density. The size of the particles forming these fractions corresponded to the upper limit of the *Percoll* particles (2.8 μm), the value of side scatter was about 15. The fluorescence intensity per particle had a mean value around 150. Fraction T/3 differed significantly from the upper two fractions. The majority of particles had the size around that of the marker beads (7.3 μm), and some of them were even bigger than thylakoids. Particles of the same size differed greatly in their side scatter which indicated different

structural features of the particles and not their aggregation or clumping. Particles of all sizes were characterized by high fluorescence intensity per particle. The mean value was around 2000 relative units but some particles reached the limit of 10 000 relative units.

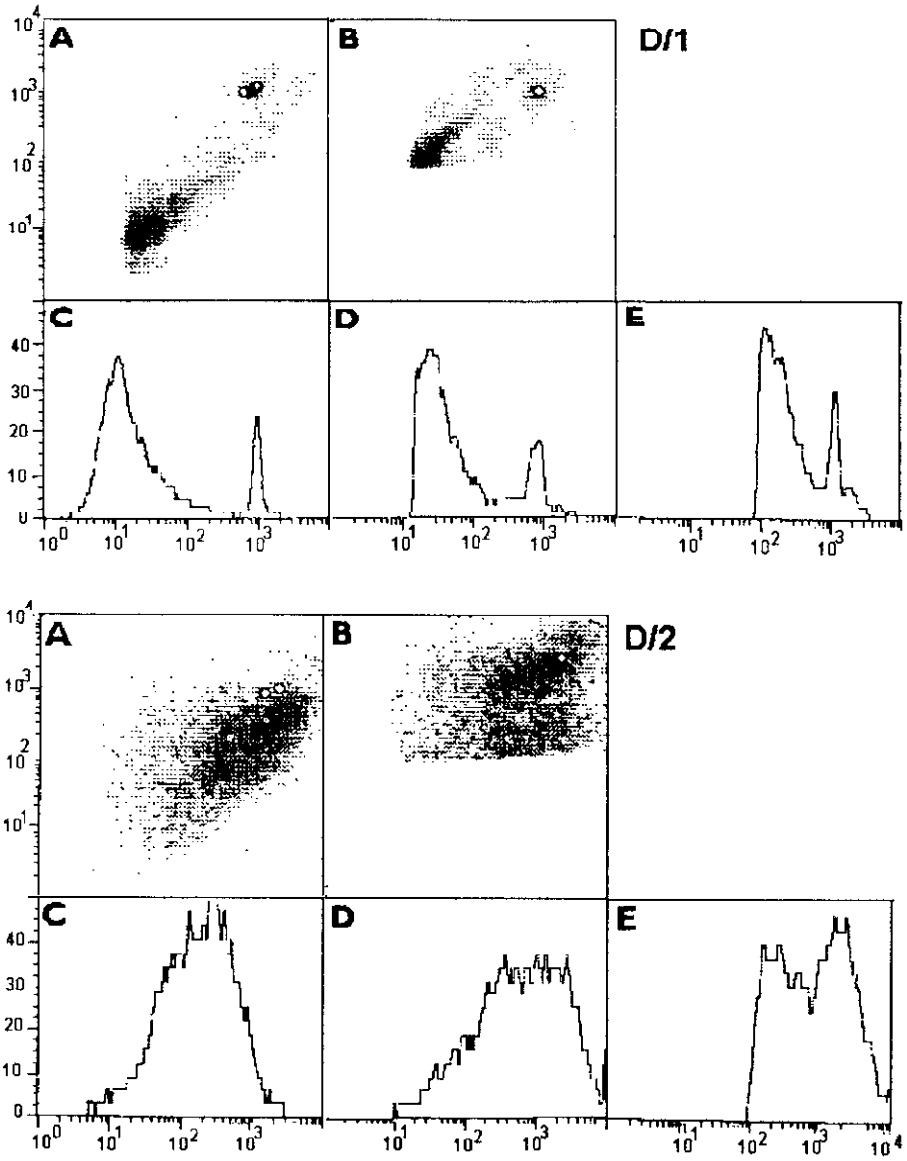

Fig. 5. Flow cytometric characteristics of digitonin fragments D/1 and D/2. For notes see Fig. 4.

The cationic detergent CPC caused a similar pattern of fragmentation as did the *Triton X-100*. Also the upper two fractions had similar flow cytometric

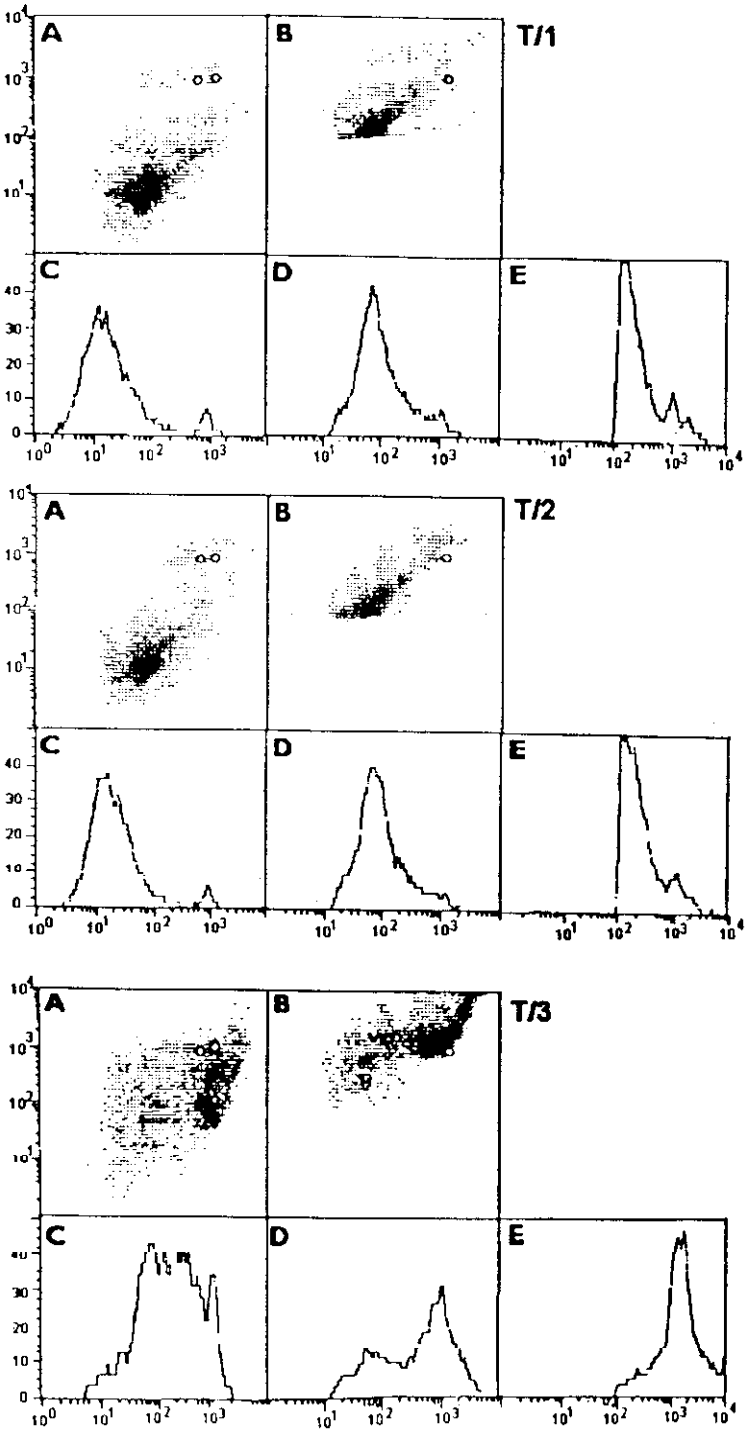

Fig. 6. Flow cytometric characteristics of *Triton X-100* fragments T/1 to T/3. For notes see Fig. 4.

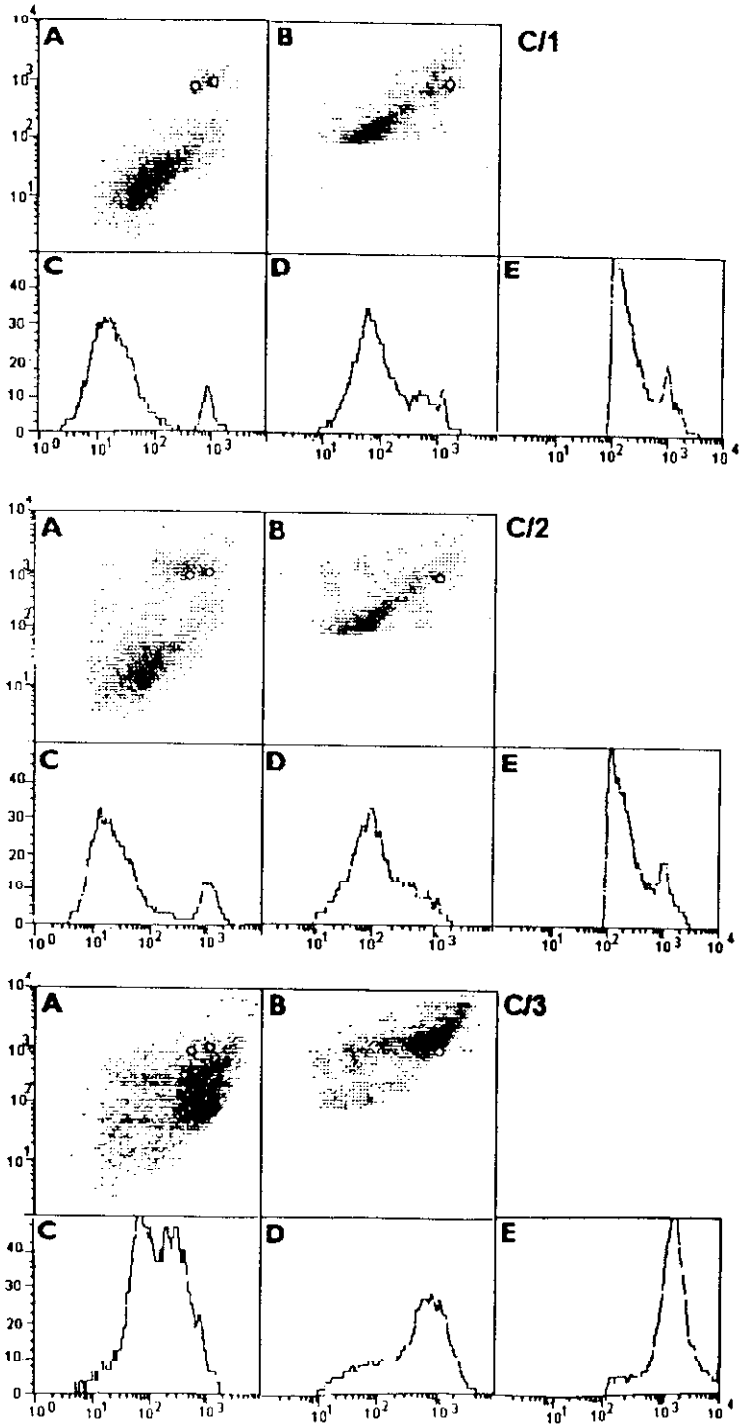

Fig. 7. Flow cytometric characteristics of cetylpyridinium chloride fragments C/1 to C/3. For notes see Fig. 4.

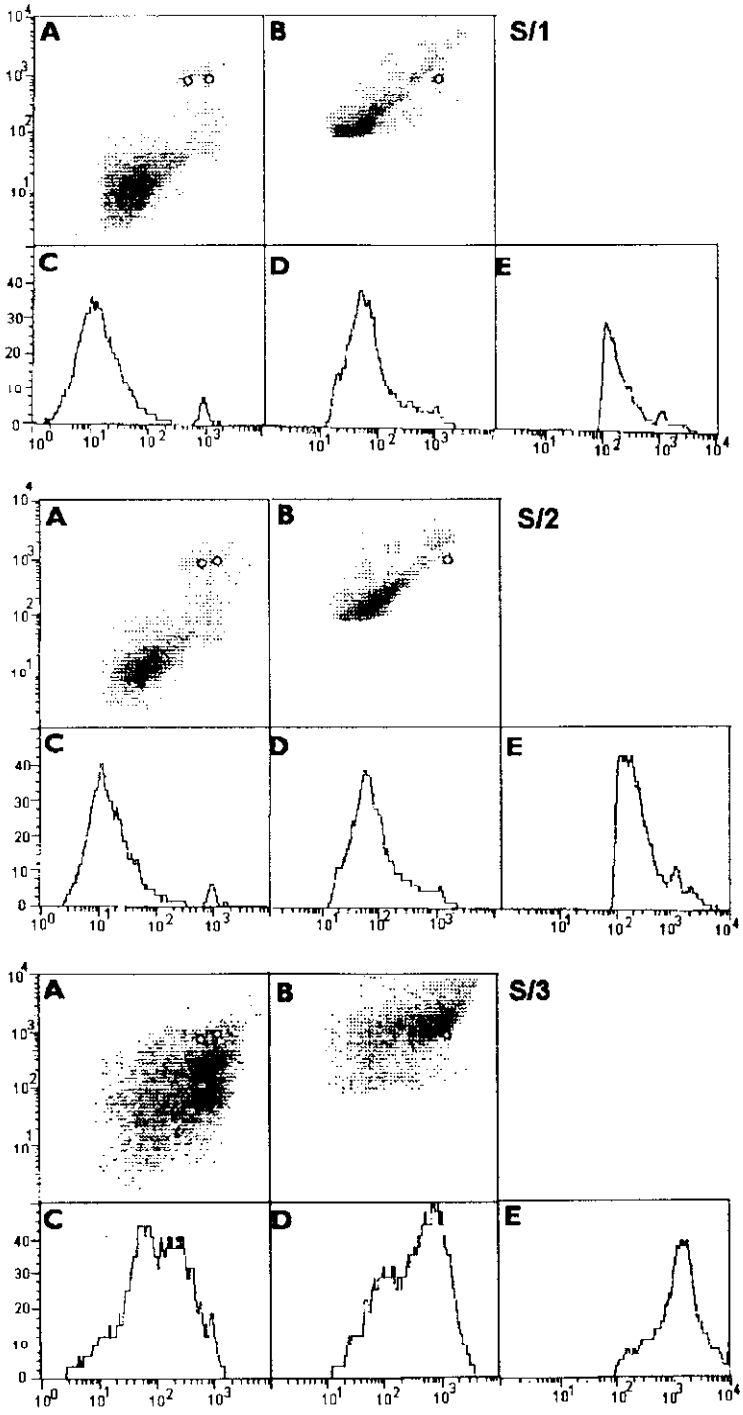

Fig. 8. Flow cytometric characteristics of SDS fragments S/1-S/3. For notes see Fig. 4.

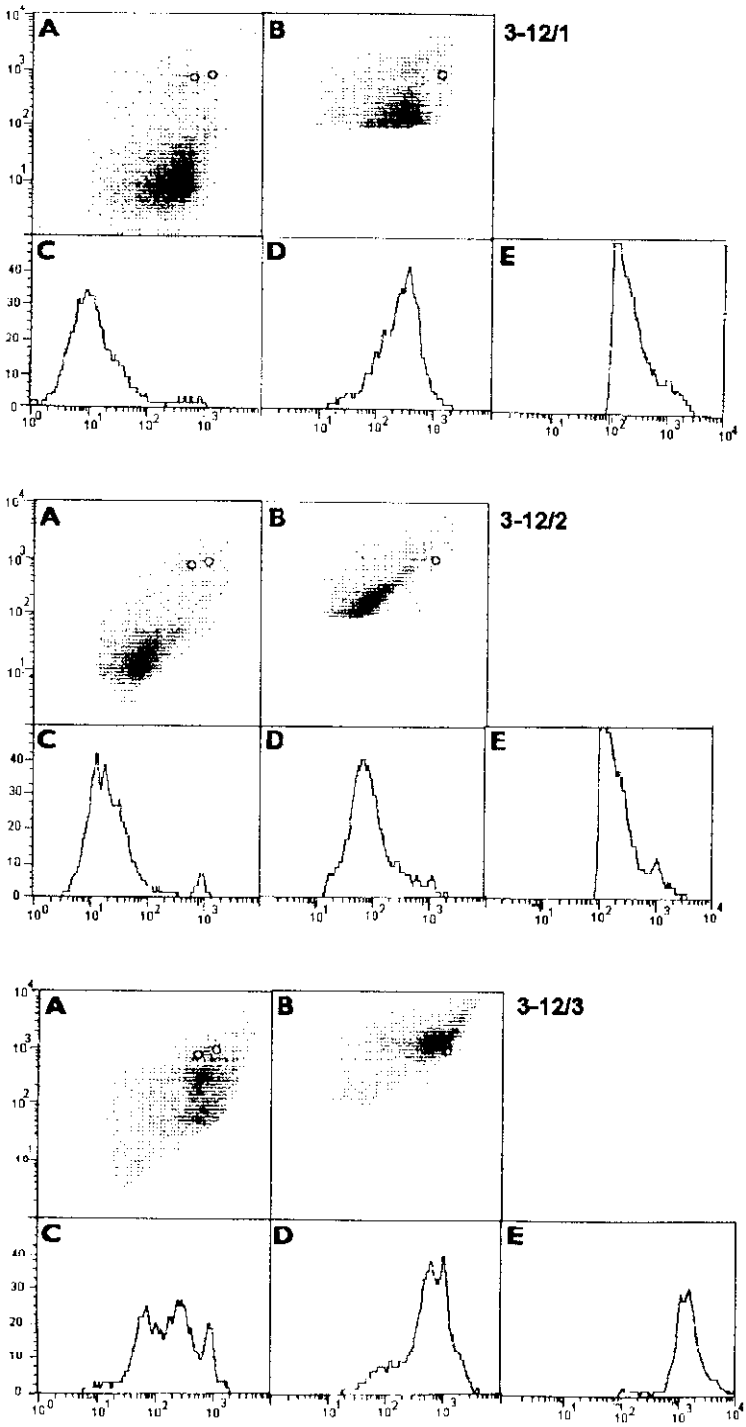

Fig. 9. Flow cytometric characteristics of *Zwittergent* 3-12 fragments 3-12/1 to 3-12/3. For notes see Fig. 4.

characteristics (Fig. 7). Their size was again on the upper edge of the size distribution of *Percoll* particles (*i.e.*, around 2.8 μm), the side scatter had a mean value around 15, and fluorescence intensity per particle had a value around 125. The fraction C/3 resembled by all its characteristics the fraction T/3. An analogous situation was found after fragmentation of the thylakoid membranes with the anionic detergent SDS (Fig. 8), the flow cytometric characteristics were almost identical to those illustrated in Figs. 6 and 7.

The zwitterionic detergent *Zwittergent* 3-12 has a 12-carbon alkyl chain and thus structurally resembles SDS. The fractionation pattern (Fig. 1) resembled the other detergents, but Fig. 9 indicates some deviations from the general pattern. Fraction 3-12/1 was composed of particles the size of which was above the size of *Percoll* particles (2.8 μm) and below the size of marker beads (4.9 μm). They were characterized by low side scatter (around 10) and low fluorescence intensity per particle - around 150 relative units. Fractions 3-12/2 and 3-12/3 had characteristics similar to the corresponding fractions formed by other detergents. The main variations observed in fractions 3 formed by various detergents consisted mainly in the ratio of populations of smaller and bigger particles as is apparent from comparison of histograms 3*D* in Fig. 9.

The zwitterionic detergent *Zwittergent* 3-14 produced a similar fractionation pattern as the other detergents besides digitonin, and individual fractions had flow cytometric characteristics similar to the corresponding fractions produced by other detergents (Fig. 10). Fraction 3-14/1 differed from other low-density fractions by the presence of population of particles with the size equivalent to the marker beads with diameter 4.9 μm. This population was also detected by the haematological analyser (Table 1) where the diameter of the particles was around 3.9 μm. These bigger particles had high fluorescence intensity per particle, *i.e.*, 1000 relative units. Fraction 3-14/2 did not possess a pronounced population of bigger particles and resembled more the corresponding fractions produced by other detergents. An analogical situation was observed in fraction 3-14/3.

The effect of zwitterionic detergent *Zwittergent* 3-16 differed from other detergents: both the upper two fractions were sharply separated and fitted by their density to the range occupied by fraction 1 in other detergents. The flow cytometric analysis (Fig. 11) showed that fraction 3-16/1 was formed by two populations of particles. The major population consisted of particles which had a smaller size than the corresponding fraction 3-14/1, but the other population had the same diameter as the corresponding population of fraction 3-14/1, *i.e.*, 4.9 μm. The size of these particles determined by haematologic analyzer was between 3.9 and 4.4 μm (Table 1). In the fraction 3-16/2 the population of bigger particles was more pronounced and contained particles even bigger than the marker beads with diameter 7.3 μm. The character of fraction 3-16/3 resembled more fraction D/2 than the corresponding fractions produced by other detergents. Some of the particles of 3-16/3 fraction were bigger than thylakoids (Fig. 4*D*). Contrary to fraction D/2, particles forming the fraction 3-16/3 had a narrower size distribution and the peak in the histogram

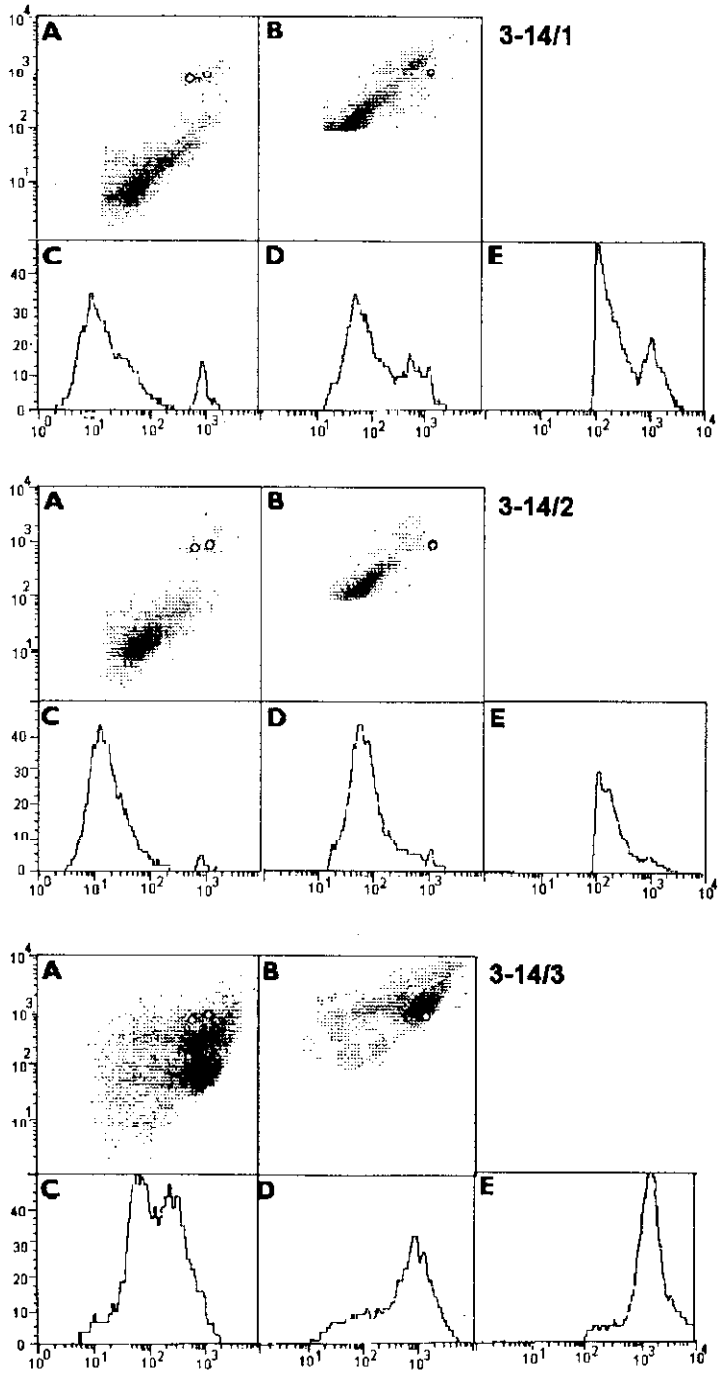

Fig. 10. Flow cytometric characteristics of *Zwittergent* 3 14 fragments 3 14/1 to 3 14/3. For notes see Fig. 4.

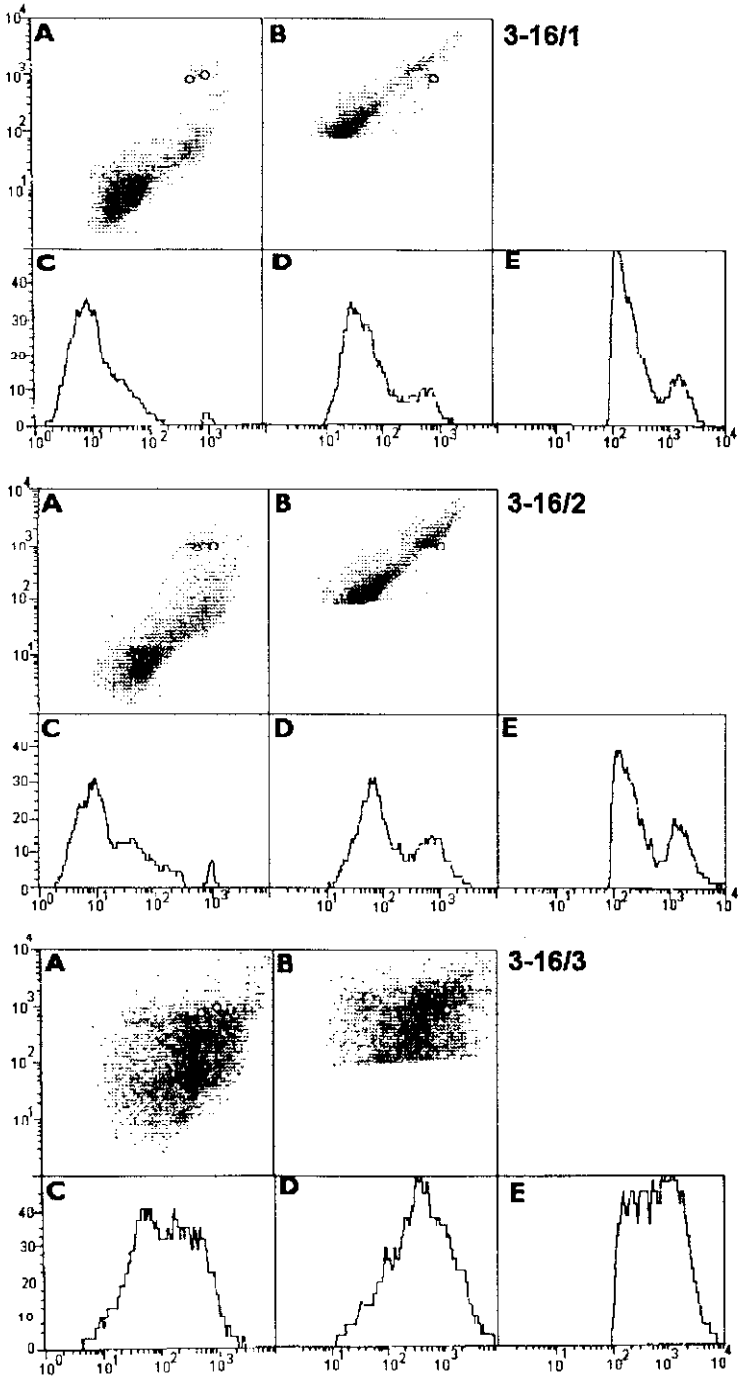

Fig. 11. Flow cytometric characteristics of *Zwittergent* 3-16 fragments 3-16/1 to 3 16/3. For notes see Fig. 4.

of forward scatter was shifted to smaller particles. On the other hand, the fraction 3-16/3 had wider distribution of side scatter which indicated its composition of particles with variable structural features. Also, in fraction 3-16/3 there was no evidence for the separation of particles into two different populations on the basis of their fluorescence, as was the case in fraction D/2. Thus, there was a continuous change in fluorescence intensity of particles of the same size.

Discussion

The detergents for thylakoid fragmentation are usually chosen empirically. Their effectiveness and fractionation pattern which they produce is influenced by many variables. We investigated the effects of detergent chemical structure on the qualities of the produced thylakoid particles with the aim to relate the size distribution to particle density. The detergents were compared according to several criteria. The effect of molecular mass and the shape of a detergent molecule was studied with non-ionic detergents *Triton X-100* and digitonin. While *Triton X-100* has high molecular mass and a bulky molecule, digitonin is of low molecular mass and planar structure. Further we studied the effects of detergent charge by comparing molecules of the same side-chain length: we used anionic sodium dodecylsulfate (12-carbon chain) in relation to 12-carbon *Zwittergent 12* (bearing both positive and negative charge), and cationic cetylpyridinium chloride (16-carbon side chain) was compared to 16-carbon *Zwittergent 16*. The effect of increasing length of side chain was studied in a group of *Zwittergents* with 12, 14, and 16 carbons in their side chain. For details of fractionation patterns by these detergents see Wilhelmová (1994).

The group of seven detergents differing in their chemical properties fragmented the thylakoid membranes in only three principally different patterns. Digitonin differed from all other detergents by forming only two fractions, the group of structurally dissimilar detergents, *Triton X-100*, CPC, SDS, *Zwittergents* 3-12 and 3-14 gave a similar fragmentation pattern, and the *Zwittergent* 3-16 produced a special fragmentation pattern. Using instruments working on different principles enabled an independent comparison of sizes of the particles creating individual fractions. The particle size was estimated by flow cytometry and by a haematologic analyzer. The correlation between these two determinations was high; only in fractions with a low number of particles the haematologic analyzer was not able to detect larger particles. The differences might consist in different conductivity properties of blood cells and thylakoid membranes, as lower values were obtained also for the calibration microbeads. The flow cytometer has a great advantage of taking into consideration only fluorescing particles thus avoiding the problem of noise of electronic measurement. Measuring the characteristics of *Percoll* particles enabled to relate the thylakoid fragments to *Percoll* as to an inner standard. Actually, there were populations of thylakoid membrane particles with a similar size distribution as the *Percoll* particles, but they were discerned on the basis of their higher side scatter and, especially, their fluorescence.

The most striking feature of detergent fractions was the presence of particle populations that had greater size than the original thylakoid membranes. This effect was not detergent-specific and indicated the way of detergent action. This might consist in a rupture of original membrane vesicles and their reconstitution into a new kind of vesicles which could be of different size. The size of newly formed vesicles would be independent of the original size. During reconstitution, some membrane components, namely proteins, may be lost and this might be the way by which detergents could influence the density of newly formed particles. An alternative explanation would consist in variable content of the detergent in particles of different density. The bigger particles (with diameters bigger than 4.9μm) tended to accumulate in the denser fractions, but after treatment with *Zwittergents* 3-14 and 3-16 they were observed also in the light fractions. It implies that the fractionation pattern under given conditions was independent of the size of the particles. Fluorescence intensity of the particles as measured by flow cytometry represented the number of fluorophores per particle. In the fragments of thylakoid membranes it corresponded to their chlorophyll concentration, especially in the particles of equal size differing by fluorescence intensity per particle. Otherwise, bigger particles would contain more chlorophyll molecules than the smaller ones and thus would possess a higher fluorescence intensity.

An interesting relationship was found between the value of side scatter and the diameter of the particles. Generally, the bigger particles had higher values of side scatter compared to smaller particles. In the blood cells the intensity of side scatter correlates with the complexity of inner structure of the cells - the presence of granules, number of organelles, *etc*. However, this situation is not encountered in the particles prepared from thylakoid membranes. We suggest that the high intensity of side scatter could be produced by formation of multi-layered concentric liposomes, as more layers would be present in particles of bigger diameter. An exception of the general pattern represented fraction 3-12/1, where the particle size approximated 4.9 μm, yet they had a very low side scatter. This could be caused by formation of single-layered liposomes. The structure of the particles could be further probed by electron microscopy.

The character of particles produced by detergents can be used to deduce the mechanisms of membrane fragmentation. For instance, the similar properties of dense fractions produced by digitonin and *Zwittergent* 3-16, and their difference from corresponding fractions produced by other detergents deserves further study. Also the observation that structurally dissimilar detergents fragment the thylakoid membrane in a standard way and produce particles of a similar nature is important. We believe that flow cytometric analysis of thylakoid membranes can be used for the determination of their structural organization and can supply important information complementary to chemical analysis.

References

Antonopoulou, P., Akoyunoglou, G.: Pigment and polypeptide composition of the light-harvesting complex of the photosystem I unit. - In: Akoyunoglou, G., Senger, H. (ed.): Regulation of Chloroplast Differentiation. Pp. 267-272. A.R. Liss, New York 1986.

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.

Camm, E.L., Green, B.R., Allred, D.R., Staehelin, L.A.: Association of the 33 kDa extrinsic polypeptide (water-spliting) with PS II particles: immunochemical quantification of residual polypeptide after membrane extraction. - Photosynth. Res. **13**: 69-80, 1987.

Hansson, O., Wydrzynski, T.: Current perceptions of Photosystem II. - Photosynth. Res. **23**: 131-162, 1990.

Herrin, D.L., Plumley, F.G., Ikeuchi, M., Michaels, A.S., Schmidt, G.W.: Chlorophyll antenna proteins of photosystem I. Topology, synthesis, and regulation of the 20-kDa subunit of *Chlamydomonas* light-harvesting complex of photosystem I. - Arch. Biochem. Biophys. **254**: 397-408, 1987.

Jursinic, P., Govindjee: The rise in chlorophyll *a* fluorescence yield and decay in delayed light emission in Tris-washed chloroplasts in the 6-100 μs time range after an excitation flash. - Biochim. biophys. Acta **461**: 253-267, 1977.

Ort, D.R.: Energy transduction in oxygenic photosynthesis: an overview of structure and mechanism. - In: Staehelin, L.A., Arntzen, C.J. (ed.): Photosynthesis III. Pp. 143-196. Springer-Verlag, Berlin - Heidelberg - New York - Tokyo 1986.

Šesták, Z., Zima, J., Wilhelmová, N.: Ontogenetic changes in the internal limitations to bean-leaf photosynthesis. 4. Effect of pH of the isolation and/or reaction medium on the activities of photosystems 1 and 2. - Photosynthetica **12**: 1-6, 1978.

Wilhelmová, N., Šesták, Z.: The effect of various detergents on the fragmentation of thylakoid membranes during leaf ontogeny. - In: Baltscheffsky, M. (ed.): Current Research in Photosynthesis. Vol. II. Pp. 931-934. Kluwer Academic Publishers, Dordrecht - Boston - London 1990.

Wilhelmová, N.: Thylakoid membrane fragmentation by means of different detergents during leaf ontogeny. - Photosynthetica **30**: 415-424, 1994.