Proceedings of The Institute of Acoustics

PHOTOACOUSTIC SPECTROSCOPY OF IMMOBILIZED THYLAKOIDS BY GLUTARALDEHYDE ACTION.

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The direct bioconversion of solar energy on a continuous basis can be used to produce high energy compounds such as ATP and hydrogen gas. It was reported (1) that chloroplasts could produce a small amount of hydrogen from water when coupled to a bacterial hydrogenase enzyme. In fact, one of the limiting steps is the stability of the chloroplast membrane (thylakoid) functions. Several laboratories, including ours, have tried to stabilize in vitro the photochemical activity of thylakoids using various immobilization methods (2,7). Especially, increases of stability after immobilization were observed by using glutaraldehyde in the presence of serum albumin at subzero temperature (4,7). Recently, the chemical action of glutaraldehyde on the structure and function of chloroplast membrane has been reviewed by Papageorgiou (8).
Photoacoustic spectroscopy (PAS), which has been already used in studies on immobilized hemoglobin by action of glutaraldehyde (9), has also proved to be a good tool for the study of the photochemical process (10,11). In this paper, the effect of glutaraldehyde on native and immobilized thylakoids is studied using PAS. The various modifications exhibited by the corresponding spectra are related to the observed oxygen production of the specimens.

MATERIALS AND METHODS

Lettuce thylakoids were obtained as already described (4,7).

(1) 0.2 ml of thylakoids (corresponding to 1 mg of chlorophyll) were mixed during 3,30 min or 2 hours with aqueous glutaraldehyde solution (2.5 % as a final concentration).

(2) Glutaraldehyde was mixed with bovine serum albumin (5.8 % as a final concentration) during 1 min and then thylakoid suspension was added during 3 or 30 min. After these two different treatments, the preparations were centrifuged and washed in Hepes - sorbitol buffer.

(3) Immobilization was performed by mixing 1.65 ml of 0.02M pH7 phosphate buffer, 1,25 ml of a 20 % bovine serum albumin solution, 0.6 ml of thylakoid and 1 ml of glutaraldehyde at 1.25%. Such a mixture was frozen at -20°C during 2 hours and then slowly thawed at 4°C and rinsed. An insoluble polymer was obtained.

O_2 evolution was measured amperometrically with a Clark-type electrode and parabenzoquinone (1mM) was used as the electron acceptor, ammoniumchloride (5mM) was used as the uncoupling agent (4,7). By using parabenzoquinone, the electron transport of photosystem II (PSII) only is measured.

The two beam photoacoustic spectrometer used was the in house-build set-up previously reported (9). The test materials were loaded thoroughly drained in the cavity (10x4x3mm) of the specimen cell. Light modulation frequency was 36 Hz. Typical time scale for scans was 74 mm/min. PA signals were sent to a HP 2649 G graphic terminal through a twelve-bit analog-digital converter.

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TABLE I-The effect of glutaraldehyde treatment on electron transport rate of thylakoids (μmoles O_2.mg chlorophyll^{-1}.hr^{-1}), and on the PA signal ratio R

GLUTARALDEHYDE	BOVINE SERUM ALBUMIN	ACTIVITY	R
No	-	170.3	0.776
No	+	219	0.873
2.5 % 3 min	-	29.9	0.94
2.5 % 30 min	-	27.8	-
2.5 % 2 hr	-	27.8	1.00
0.32 % 3 min	+	92.6	0.845
0.32 % 30 min	+	87.3	0.88

TABLE II-The effect of immobilization on electron transport rate and on the PA signal ratio R

GLUTARALDEHYDE CONCENTRATION (%)	ACTIVITY	R
0.20	38	0.68
0.32	27.2	0.73
0.5	22	0.75

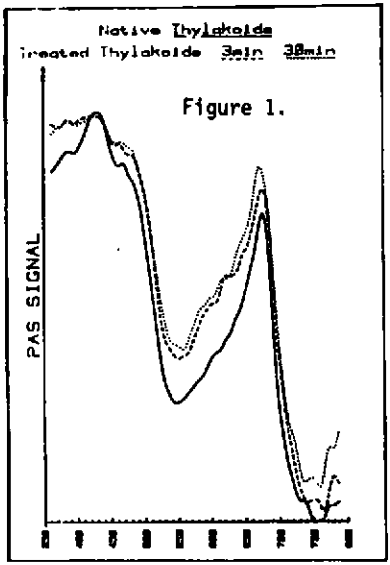

Figure 1.

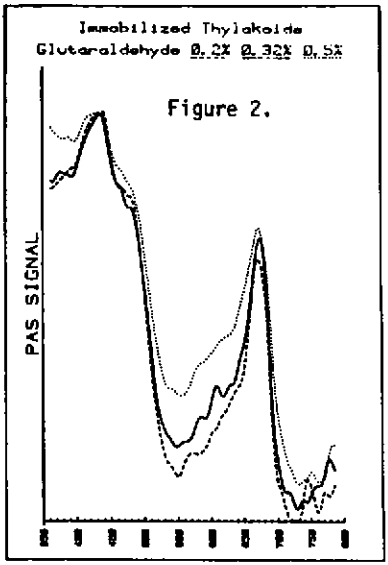

Figure 2.

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The number of points used was usually 380. After a smoothing (based on the Blackman curve) consisting in a shifting average on twenty-one weighted points, the spectra were printed with a HP 9872A plotter.

RESULTS AND DISCUSSION

The activity results shown in Table I indicate that preincubation of thylakoids suspension with 2.5 % glutaraldehyde, as a final concentration, during various times, affects considerably the oxygen production. It seems that such inhibition occurs during the first minutes of the treatment. PA signals are obtained from these samples, and as already described by Cahen et al (10) the corresponding spectra can be normalized at 440 nm to facilitate the comparison between the different samples (Figure 1). At 680 nm the treated thylakoids exhibit a signal higher than that of the native ones. The ratios of the PA signal at 670-680 nm to the signal at 440 nm (R) are given in Table I. The ratio increase is related to the decrease of the corresponding activities. When thylakoids are inactivated, an increase of heat emmission is observed. Furthermore, it can be noted also an increase of the PA signal in the 350-420 nm interval for the treated specimens (Figure 1).

Generally the inhibition of photosynthetic activity can be caused by the release of lipoprotein particles and of the free fatty acids (8). As already described (4,7) the Table I shows that excellent protection of electron transport rate is achieved by preincubation with serum albumin (see Materials and Methods §2). However an increase of PA signal ratio is already observed suggesting a peculiar effect of albumin on the non radiative deexcitation processes.

In these experiments, the glutaraldehyde concentration has been chosen to be compared to the concentrations used in immobilized systems.

In terms of functional thylakoid stability immobilization offers some advantages in the case of continuous work (4,7). It has been shown previously that Scanning Electron Microscopy of immobilized cells with different glutaraldehyde concentrations gives morphological differences. Especially, the structural integrity of the immobilized cells is preserved with 0.3-0.4 % glutaraldehyde. In the present case, the activities are given in Table II.As compared to native thylakoids of the Figure 1, the spectrum corresponding to immobilized thylakoids (Figure 2) with 0.32 % of glutaraldehyde presents no significant differences.

The respective ratio (R) between signals at 680 nm and 440 nm increases also as a function of glutaraldehyde (Table II) but the obtained values are quite lower than those presented in Table I (0.7 vs 0.9). The absence of modification in the 350 - 420 nm interval for the polymer obtained with 0.32 % of glutaraldehyde should be noted also. However the activities measured after immobilization indicate a deleterious effect of glutaraldehyde. The results in continuous working already reported (7) indicate a high stabilization for the immobilized thylakoids. The different absolute values of R shown on Table I and II may be used to appreciate (0.7 vs 0.9) the stabilization effect.Thus both treatments (chemical modification and immobilization process) are quite different. In terms of activity and stability the use of PAS in the study of immobilized active photosystems has proved valuable, since on the one hand it confirms the kinetic observations, and gives on the other hand additional information on the physical properties of the immobilized photosystems.

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REFERENCES

1. BENEMANN,J.R., MIYAMOTO,K. and HALLENBECK,P.C. (1980) Enzyme Microb. Technol. 2 103-111
2. KITAJIMA,M. and BUTLER,W.L. (1976) Plant Physiol. 57 746-750
3. OCHIAI,H., SHIBATA,H., MATSUO,T., HASCHINOKUCHI,K. and INAMURA,I. (1978) Agric. Biol. Chem. 42 683-685
4. COCQUEMPOT,M.F., THOMAS,D., CHAMPIGNY,M.L. and MOYSE,A. (1979) European J. Appl. Microbiol. Biotechnol. 8 37-41
5. GISBY,P.E. and HALL,D.O. (1980) Nature 287 251-253
6. KARUBE,I., OTSUKA,T., KAYANO,H. MATSUNAGA,T. and SUZUKI,S. (1980) Biotechnol. Bioeng. 22 2655-2665
7. COCQUEMPOT,M.F., THOMASSET,B., BARBOTIN,J.N., GELLF,G. and THOMAS,D. (1981) European J. Appl. Microbiol. Biotechnol. (in press)
8. PAPAGEORGIOU,G.C. (1980) Methods Enzymol. 69 613-625
9. VEJUX,A.M. and BAE,P. (1980) J. Opt. Soc. Am. 70 560-562
10. CAHEN,D., MALKINS,S. and LERNER,E.I. (1978) FEBS Letters 91 339-342
11. CAHEN,D., BULTS,G., GARTY,H. and MALKIN,S. (1980) J. Biochem. Biophys. Methods 3 293-310
12. BARBOTIN,J.N. and THOMASSET,B. (1980) Biochimie 62 359-365