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RATE LIMITING FACTORS IN LEAF PHOTOSYNTHESIS

II. ELECTRON TRANSPORT

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Increased scattering of a weak 535 nm measuring beam which indicates the light-dependent formation of a transthylakoid proton gradient in leaves was used to examine the role of the electron-transport chain in limiting photosynthetic carbon assimilation. The proton gradient is supported by electron flux and indicates thylakoid energization. In CO_2-free air, half saturation of thylakoid energization was observed at intensities of red light ranging from 2 to 50 $W \cdot m^{-2}$ in different plant species. The differences were attributed to different carbohydrate availability for energy-consuming photorespiratory processes when external CO_2 was absent. Thylakoid energization of shade leaves *(Asarum, Fagus)* was saturated at lower light intensities than that of sun leaves *(Phaseolus, Fagus)*. When photorespiratory carbohydrate oxidation was suppressed by decreasing the O_2 concentration from 21 to 2% in the absence of CO_2, thylakoid energization saturated at lower light intensities than in CO_2-free air. CO_2 decreased thylakoid energization particularly at low light intensities. Under high intensity illumination, however, thylakoid energization was remarkably high even in the presence of saturating CO_2. Apparently, electron transport was capable of maintaining the energy status of the photosynthetic apparatus at a high level even when photosynthetic carbon fluxes were maximal. This suggests that electron transport is less important in limiting photosynthesis than previously thought.

Introduction

When light intensities are very high, leaf photosynthesis may be limited by the availability of CO_2 or by the catalytic capacity of the photosynthetic apparatus to convert substrates to final products. Actually, interaction of several partial limitations is likely to determine the maximum photosynthetic performance of a leaf. It is well known that photosynthetic CO_2 fixation is enhanced by increasing CO_2 concentrations under conditions of high intensity illumination. The question remains what the limiting factors are in this situation. In the previous publication [16] no evidence was obtained for a significant rate-limiting function of the enzyme systems responsible for reduction of 3-phosphoglycerate, the conversion of the resulting triosephosphate to fructose bisphosphate and the dephosphorylation of this metabolite to fructose 6-phosphate. In the reaction sequence from 3-phosphoglycerate to fructose 1,6-bisphosphate, activities of involved enzymes were so high that fluxes were driven by very small gradients in free energy and still larger fluxes appeared easily possible. Ribulose 1,5-bisphosphate concentrations, on the other hand, remained in the millimolar range even when CO_2 was saturating, suggesting that the activity of ribulose 1,5-bisphosphate carboxylase still limited photosynthesis even under CO_2 saturation. Moreover, the regeneration of ribulose 1,5-

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bisphosphate from hexosephosphate and triosephosphate also appeared to be a factor in limiting flux in the Calvin cycle at high CO_2 concentration and high light intensities. The conclusion that the Calvin cycle limits photosynthesis under high light intensity illumination even when CO_2 concentrations are high is in some conflict with the belief that electron transport is the main limiting factor of photosynthesis under these conditions. This assumption is based on the observation that the high rates of electron transport to CO_2 measured in vivo correspond to or exceed maximum rates of uncoupled electron transport to ferrycyanide or methyl viologen in vitro. However, such comparison of rates has been misleading in the past in several instances, as in the case of ribulose 1,5-bisphosphate carboxylase or fructose 1,6-bisphosphatase, and the rates of electron transport in vitro depend very much on experimental conditions [1].

In this communication, we use light scattering of leaves to examine the question to which extent electron transport may limit photosynthesis in vivo.

Materials and Methods

Spinach (*Spinacia oleracea* L., Yates Hybrid 102) was grown in a greenhouse; *Fagus sylvatica* L., *Asarum europaeum* L., *Phaseolus vulgaris* L. ssp. nanus Asch. and *Lycopersicum esculentum* Mill. were grown in the Botanical Garden. Leaves were harvested during February (*Spinacia*), September and October.

CO_2 gas exchange and scattering of a weak green measuring beam by the leaves were measured as described earlier [2,3]. The difference between light scattering, measured by recording apparent absorption of the leaves, in the dark and in the light was taken as a relative measure for energization of the photosynthetic apparatus [2,4].

Cytochrome *f* was measured in isolated chloroplasts as described by Heber et al. [5].

Results and Discussion

Transthylakoid proton gradient and membrane potential in leaves

During chloroplast electron transport, a proton gradient and an electrical gradient are established

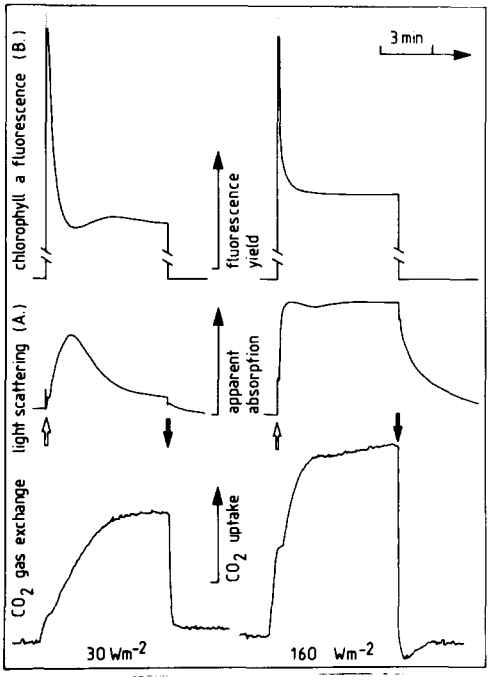

Fig. 1. Traces of CO_2 gas exchange, light scattering (A) and chlorophyll *a* fluorescence (B) by a spinach leaf at a low (30 $W \cdot m^{-2}$) and a high (160 $W \cdot m^{-2}$) light intensity in air (330 $\mu l \cdot l^{-1}$ CO_2 and 21% O_2). The fluorescence signals produced by illumination with 30 and 160 $W \cdot m^{-2}$ were measured at differnt sensitivities of the recorder. Arrows indicate illumination and darkening.

across the thylakoid membranes. These gradients represent the driving force for ATP synthesis [6]. They are discharged when ATP is consumed during carbon reduction, because ATP must be resynthesized to maintain a proper balance between ATP and ADP. ATP synthesis is linked to proton efflux from the intrathylakoid space. Only when ATP accumulates, i.e., when the ratio of ATP to ADP is high, or when the capacity of the ATP synthetase of the thylakoid membrane is over-taxed and, therefore, this enzyme becomes rate-limiting for ATP synthesis, can proton and electrical gradients remain large in the light. The magnitude of these gradients can therefore be used to assess the interaction between electron transport and carbon metabolism in photosynthesis.

In leaves, the proton gradient cannot be measured directly. However, formation of the proton gradient gives rise to altered scattering of light by the thylakoid membranes [2]. Light scattering

increases as the proton gradient increases. In intact chloroplasts, a correlation has been established between proton gradient and light scattering [7]. Moreover, since in many instances the magnitude of the proton gradient appears to be related to the ratio of ATP to ADP [4], light scattering may be used to gain information on the state of the chloroplast ATP/ADP ratio or of the phosphorylation potential (ATP)/(ADP)(P_i). Even in work with leaves, light scattering has been found to be maximal when ATP to ADP ratios were high, and light scattering was low when ATP synthesis was inhibited by nitrogen, or when ATP consumption by the carbon reduction cycle was high [4]. It should be noted that the water potential of the leaves needs to be kept constant during the light scattering measurements.

In chloroplast ATP synthesis, the free energy gradient represented by the light-generated membrane potential is small compared with that stored in the proton gradient (For review see Ref. 8). Fig. 1 shows simultaneous recordings of photosynthesis, chlorophyll a fluorescence and apparent absorbance at 535 nm of a spinach leaf at two different light intensities. While the rate of photosynthetic CO_2 uptake was very low during induction in low light, light scattering which displays slow kinetics (trace A) was initially high because the proton gradient could not be discharged. It decreased as ATP was consumed when photosynthesis accelerated. The fast signals in trace A seen on illumination and darkening may be taken to indicate formation and decay of the membrane potential. The fast 518 nm absorbance change is broad and is still considerable at 535 nm. It is apparent that the electrical gradient is smaller in the steady state of photosynthesis than during the onset of illumination where it increases rapidly and then exhibits a transient collapse in response to ion movements across the thylakoid membrane. Chlorophyll *a* fluorescence (trace B) is shown for comparison. Close to the steady state, fluorescence is low when light scattering is high, and vice versa. Low fluorescence is a consequence of energy-dependent fluorescence quenching [10]. However, fluorescence is a more complex indicator of thylakoid energization than light scattering because it is also influenced by the redox state of the electron-transport chain which determines fluorescence yield, particularly in the initial phase of illumination [9,10]. Under high intensity illumination where photosynthesis was light-saturated, both light scattering and the steady-state membrane potential were much larger than during low intensity illumination. Fig. 2 shows the rate of photosynthetic CO_2 fixation, light scattering and the membrane potential as a function of light intensity. Under conditions where CO_2 was removed from the gas stream which passed over the leaf, thylakoid energization as indicated by light scattering or the 518 nm signal rapidly approached light saturation as the light intensity was increased. In the presence of 330 $\mu l \cdot l^{-1}$ CO_2, both light scattering and the membrane potential were decreased and there was a sigmoidal relationship between thylakoid energization and light intensity which was caused by energy consumption during carbon assimilation. Since there is a good correlation between proton gradient as revealed by slow light scattering and the membrane potential at

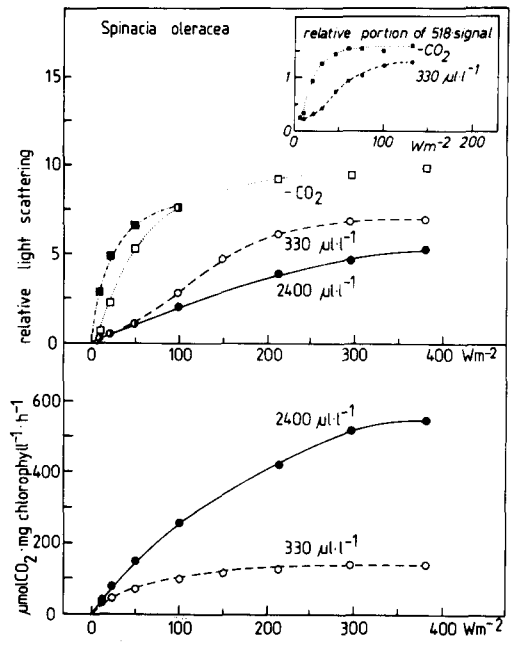

Fig. 2. Light scattering, 518 nm signal (upper part of figure) and CO_2 gas exchange (lower part) of a spinach leaf as a function of the light intensity in air containing different CO_2 concentrations (●, ○, □) or in 2% O_2/98% N_2 (without CO_2, ■). The inset shows the relative portion of the fast absorption change seen when the light is turned off. It is attributed to the electrochromic shift.

least as long as electron transport rates were significant (the relationship did not always hold in CO_2-free air and was broken when in the absence of CO_2 a reduction in oxygen concentration from 21 to 2% O_2 decreased electron transport further by minimizing photorespiratory carbon oxidation), only light scattering will be considered.

Thylakoid energization by electron transport during carbon reduction

Figs. 3–6 show light scattering and photosynthetic CO_2 fixation from different C_3 plants, with and without CO_2 present, as a function of light intensity. When CO_2 was removed from the gas stream, half-maximum light scattering was observed in bean leaves at about 50, in tomato leaves at about 30, in sun leaves of beech at 25, in spinach leaves (grown in a greenhouse) at about 20, in leaves of the shade plant *Asarum* at 15 and in shade leaves of beech at about 2 Wm^{-2} red light. Different leaves of the same species varied somewhat in their light scattering response to light. In the absence of CO_2, the differences are likely to be caused by differences in the availability of carbohydrates which are oxidized by energy-requiring photorespiratory reactions. This energy consumption during photorespiratory carbohydrate oxidation decreased the proton gradient. When the oxygen concentration was diminished from 21 to 2% in the absence of CO_2 to eliminate photorespiratory reactions, light intensities necessary for half-maximum light scattering decreased considerably (Figs. 2, 5a, 6a). It appears that there is a correlation between the nutritional status of the leaves and their capability to consume energy by carbohydrate oxidation in photorespiratory pathways when CO_2 is absent from the gas stream.

In the presence of 330 $\mu l \cdot l^{-1}$ CO_2, light-saturated rates of photosynthesis were between 20 (*Asarum*) and 140 μmol CO_2 reduced/mg chlorophyll per h (*Spinacia*). Light scattering was drastically decreased in the presence of CO_2 at low light intensities, but not necessarily at high light intensities. In been leaves and in shade leaves of beech (Figs. 6a, 3), thylakoid energization as revealed by light scattering was as extensive under high intensity illumination in the presence of air levels of CO_2 as in the absence of CO_2. In *Asarum*, stimulation of electron transport by CO_2 even increased light scattering, when the CO_2 concentration was increased (Fig. 4). In spinach (Fig. 2) and tomato (Fig. 5), air levels of CO_2 decreased leaf energization even at saturating light intensities. However, it should again be noted that there was some variability in the response of individual leaves to CO_2, and the decrease in light scattering brought about by 330 or 400 $\mu l \cdot l^{-1}$ CO_2 was occasionally only small at high light intensities.

When CO_2 was increased from air levels to CO_2 saturation, maximum rates of photosynthesis usually increased by a factor of 3–4, and higher light

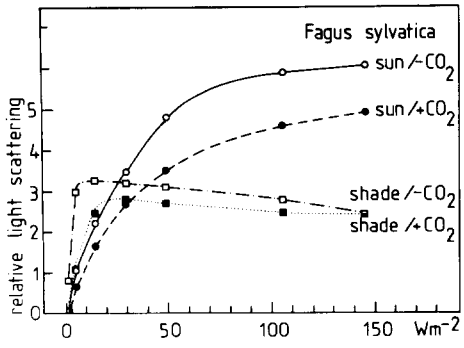

Fig. 3. Light scattering of sun (○, ●) and shade (□, ■) leaves of *Fagus sylvatica* in dependence of light intensity in air with 330 $\mu l \cdot l^{-1}$ CO_2 (closed symbols) and without CO_2 (open symbols).

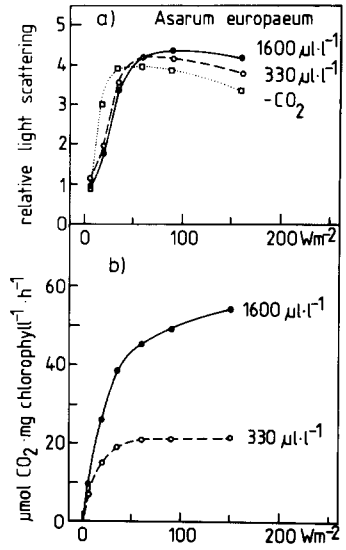

Fig. 4. Light scattering (a) and CO_2 gas exchange (b) of leaves of *Asarum europaeum* as a function of the light intensity in air with 1600 $\mu l \cdot l^{-1}$ (●), 330 $\mu l \cdot l^{-1}$ (○) and without CO_2 (□).

intensities were needed to saturate photosynthesis than in air. Under these conditions, a further significant suppression of light scattering was usually observed, particularly at high light intensities (however, see Fig. 4a with the shade plant *Asarum* for an exception). In a tomato leaf (Fig. 5a), the decrease in light scattering caused by 2400 $\mu l \cdot l^{-1}$ CO_2 was not much larger than that caused by air levels of CO_2. In a bean leaf, thylakoid deenergization by 1600 $\mu l \cdot l^{-1}$ CO_2 was comparable to that caused by 1% CO_2 (not shown) and significantly larger than that caused by air. Similar results were obtained for spinach (Fig. 2).

In beans, spinach and sun leaves of beech thylakoid energization increased as light intensity was increased until saturation was reached. In the shade leaves of *Asarum* and *Fagus*, on the other hand, thylakoid energization as indicated by light scattering was maximal at intermediate light intensities and decreased while the rate of CO_2-saturated CO_2 fixation still increased with light intensity. This was seen both in the presence of air levels of CO_2 and under conditions of CO_2 saturation.

The very high rates of CO_2 reduction observed in spinach (up to 550 μmol CO_2 reduced/mg chlorophyll per h) require comment. In electron transport, oxidation of plastoquinone is commonly

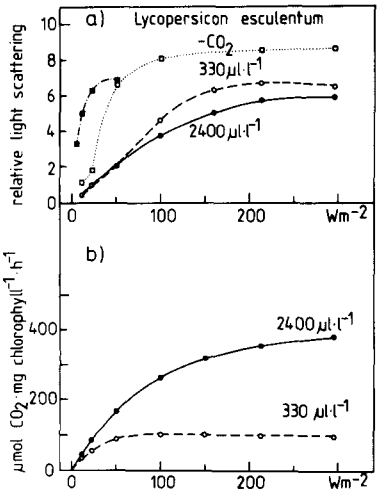

Fig. 5. Light scattering (a) and CO_2 gas exchange (b) of leaves of *Lycopersicum esculentum* as a function of the light intensity in air with 2400 $\mu l \cdot l^{-1}$ (●), 330 $\mu l \cdot l^{-1}$ (○) and without CO_2 (□), and with 2% O_2/98% N_2 (■).

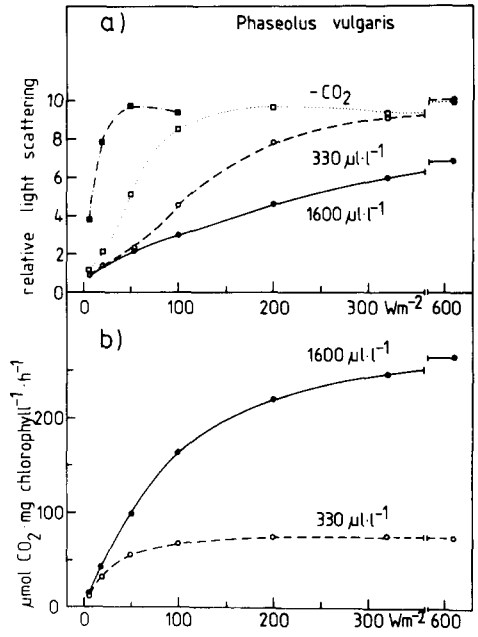

Fig. 6. Light scattering (a) and CO_2 gas exchange (b) of leaves of *Phaseolus vulgaris* in dependence of light intensity in air with 1600 $\mu l \cdot l^{-1}$ (●), 330 $\mu l \cdot l^{-1}$ (○) and without CO_2 (□), and with 2% O_2/98% N_2 (■).

considered to be rate limiting [11,12]. In flash experiments with uncoupled electron transport in chloroplasts, transfer times for an electron from plastoquinone to cytochrome *f* were reported to be between 10 and 45 ms in dependence of the redox state of the plastoquinone pool [13]. To compare such data with leaf photosynthesis, cytochrome *f* was measured in chloroplasts isolated from spinach leaves which had exhibited a capacity for 550 μmol CO_2 fixed/mg chlorophyll per h under CO_2 and light saturation. The chloroplasts contained 3.3 nmol cytochrome *f*/mg chlorophyll. The transfer time for an electron from plastoquinone to cytochrome *f* was calculated from this to be 5 ms under CO_2 and light saturation. Transfer times of 10 ms were common. Transfer is therefore faster in vivo in coupled chloroplasts than thought possible for uncoupled chloroplasts in vitro. Even this very fast electron transfer is unlikely to limit photosynthetic CO_2 fixation in vivo (cf. preceding publication [16]. Rather, even under CO_2 saturation, a major limitation to CO_2 assimilation appears to reside in the Calvin cycle when light intensities are very high. The large differences in the rate of

photosynthesis seen when the concentration of CO_2 is increased from air levels to saturation contrasts with the rather small decrease of thylakoid energization as revealed by light scattering. When electron transport limits photosynthesis, as is clearly the case at low light intensities, thylakoid energization is effectively suppressed by CO_2. The considerable energization observed in the presence of saturating CO_2 in high light makes it difficult to accept the proposition that the electron-transport chain is the main factor limiting photosynthetic fluxes when CO_2 is abundant. High thylakoid energization can be maintained only if there is a limitation to energy drainage. According to the data presented in the previous publication, this limitation may reside in the Calvin cycle. Alternatively, a limitation posed by the capacity of the thylakoid-coupling factor CF_1 to catalyze ATP synthesis must be considered. Rates of CO_2 assimilation between 300 and 600 μmol CO_2 reduced/mg chlorophyll per h require ATP turnover of about 1000–2000 μmol/mg chlorophyll per h. Avron [14] has reported rates of photophosphorylation of about 2500 μmol/mg chlorophyll per h. Since the ATP/ADP ratio observed in leaf chloroplasts under CO_2 saturation and in high light (see preceding publication) is considerably higher than that in the dark (ATP/ADP ratios in the dark in comparable experiments are below 1), and since the maximum rates of photophosphorylation measured in vitro are higher than the rates of ATP-synthesis needed to allow maximum rates of CO_2 fixation, the activity of ATP synthetase in spinach chloroplasts is unlikely to be a major factor in limiting photosynthesis.

Conclusions

Light scattering data of different plant species suggest that the electron-transport chain of leaves does not play a direct role in limiting photosynthetic flux when light intensities are very high and CO_2 is rate-saturating. In a previous communication, it has been shown that 3-phosphoglycerate reduction, the condensation of triosephosphates to fructose 1,6-bisphosphate and dephosphorylation of fructose 1,6-bisphosphate to fructose 6-phosphate could easily cope even with maximum turnover rates of the carbon reduction cycle. However, the thermodynamic gradient required to drive regeneration of ribulose 1,5-bisphosphate from hexosephosphate and triosephosphate increased considerably with increasing flux in the Calvin cycle, suggesting some rate limitation in ribulose 1,5-bisphosphate regeneration. Still, even at maximum rates of photosynthesis, high ribulose 1,5-bisphosphate levels were measured in vivo. Apparently, ribulose 1,5-bisphosphate carboxylase retained a major rate-limiting function in photosynthesis even when CO_2 and ribulose 1,5-bisphosphate, the substrates, were readily available. However, it should be noted that electron transport may play a less than direct role in limiting photosynthetic flux under conditions optimal for photosynthesis. When photosynthetic rates increased with increasing CO_2 concentrations at high light intensities, the ratio of 3-phosphoglycerate to triosephosphate was observed to increase and the ratios of ATP to ADP and of NADPH to NADP to decrease. These changes in metabolites are expected to affect metabolic regulation in the Calvin cycle [15]. Under physiological conditions, interaction of different potential rate-limiting steps will determine photosynthetic output.

Acknowledgements

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References

1 Good, N., Izawa, S. and Hind, G. (1966) Curr. Top. Bioenerg. 1, 75–112
2 Heber, U. (1969) Biochim. Biophys. Acta 180, 302–319
3 Dietz, K.-J. and Heber, U. (1983) Planta 158, 349–356
4 Kobayashi, Y., Köster, S. and Heber, U. (1982) Biochim. Biophys. Acta 682, 44–54
5 Heber, U., Boardman, N.K. and Anderson, J.M. (1976) Biochim. Biophys. Acta 423, 275–292
6 Mitchell, P. (1966) Biol. Rev. Cambridge 41, 445–502
7 Köster, S. and Heber, U. (1982) Biochim. Biophys. Acta 680, 88–94
8 Jagendorf, A.T. (1977) in Encyclopedia of Plant Physiol (Trebst, A. and Avron, M., eds.), Vol. 5, pp. 307–337, Springer Verlag, Berlin
9 Duysens, L.N.M. and Sweers, H.E. (1963) in Studies on Microalgae and Photosynthetic Bacteria (Jap. Soc. Plant Physiol., eds.), pp. 353–373, University of Tokyo Press, Tokyo
10 Butler, W.L. (1977) in Encyclopedia of Plant Physiol (Trebst,

A. and Avron, M., eds.), Vol. 5, pp. 149–167, Springer Verlag, Berlin
11 Stiehl, H.H. and Witt, H.T. (1969) Z. Naturforsch. 24 b, 1588–1598
12 Haehnel, W. (1973) Biochim. Biophys. Acta 305, 618–631
13 Schmidt-Mende, P. and Witt, H.T. (1969) Z. Naturforsch. 23b, 228–235
14 Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272
15 Gardemann, A., Stitt, M. and Heldt, H.W. (1983) Biochim. Biophys. Acta 722, 51–60
16 Dietz, J.-J. and Heber, U. (1984) Biochim. Biophys. Acta 767, 432–443