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INHIBITION OF ENERGY-TRANSDUCING FUNCTIONS OF CHLOROPLAST MEMBRANES BY LIPOPHILIC IRON CHELATORS

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SUMMARY

Lipophilic metal chelators inhibit various energy-transducing functions of chloroplasts. The following observations were made.

1. Photophosphorylation coupled to any known mode of electron transfer, i.e. whole-chain noncyclic, the partial noncyclic Photosystem I or Photosystem II reactions, or cyclic, is inhibited by several lipophilic chelators, but not by hydrophilic chelators.

2. The light- and dithioerythritol-dependent Mg^{2+}-ATPase was also inhibited by the lipophilic chelators.

3. Electron transport through either partial reaction, Photosystem I or Photosystem II was not inhibited by lipophilic chelators. Whole-chain coupled electron transport was inhibited by bathophenanthroline, and the inhibition was not reversed by uncouplers. The diketone chelators diphenyl propanedione and nonanedione inhibited the coupled, whole-chain electron transport and the inhibition was reversed by uncouplers, a pattern typical of energy transfer inhibitors.

The electron transport inhibition site is localized in the region of plastoquinone → cytochrome *f*. This inhibition site is consistent with other recent work (Prince et al. (1975) *FEBS Lett.* 51, 108 and Malkin and Aparicio (1975) *Biochem. Biophys. Res. Commun.* 63, 1157) showing that a non-heme iron protein is present in chloroplasts having a redox potential near +290 mV. A likely position for such a component to function in electron transport would be between plastoquinone and cytochrome *f*, just where our data suggests there to be a functional metalloprotein.

4. Some of the lipophilic chelators induce H^+ leakiness in the chloroplast membrane, making interpretation of their phosphorylation inhibition difficult. However, 1–3 mM nonanedione does not induce significant H^+ leakiness, while inhibiting ATP formation and the Mg^{2+}-ATPase. Nonanedione, at those concentrations, causes a two- to four-fold increase in the extent of H^+ uptake.

Abbreviations: BP, 4,7-diphenyl-1,10-phenanthroline; EDTA, ethylendiaminetetraacetic acid; Tiron, 4,5-dihydroxy-m-benzendisulfonic acid; TTFA, 4,4,4-trifluoro-1-(2-thienyl)-1,3, butanedione; DPPD, 1,3-diphenylpropanedione; CCCP, carbonyl cyanide 3-chlorophenylhydrazone.

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5. These results are consistent with, but do not prove, the involvement of a non-heme iron or a metalloprotein in chloroplast energy transduction.

INTRODUCTION

Lipophilic chelators have recently been shown to have interesting effects on mitochondrial, chloroplast and *Escherichia coli* membrane-bound ATPases and electron transport. Using the lipophilic chelator, 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline, BP), Phelps et al. [1] found inhibition of coupled mitochondrial electron transport. Three inhibition sites were found corresponding to the three energy-coupling sites, and the inhibition was prevented by uncouplers. Electron transport and the ATPase of *E. coli* [2] were inhibited by lipophilic chelators.

In the chloroplast system, lipophilic chelators such as BP and TTFA inhibited photophosphorylation and the coupled electron flow [3, 4] and the Mg^{2+}-dependent ATPase [4]. The Ca^{2+}-dependent ATPase of the solubilized coupling factor was not inhibited by chelators [4].

The inhibition of electron transport by metal chelators is of interest in light of recent reports of an electron spin resonance-detected non-heme iron signal in chloroplasts characterized by a redox potential near +290 mV [5]. A similar ESR signal was observed in photosynthetic bacteria [6]. Such signals are hypothesized to be due to an iron-sulfur protein of the "Rieske type" found in the mitochondrial electron transport chain [7]. If there is a protein of this type in the chloroplast electron transport chain having a redox potential near +290 mV, its functional location, based on the observed redox potential, would be between plastoquinone and cytochrome *f*. If the previously reported electron transport inhibition [3, 4] by chelators is due to effects on such a protein then the location of the inhibitory site can be pinpointed using Photosystem I and Photosystem II partial electron transport assay systems. Experiments of this nature are reported below.

Bathophenanthroline and DPPD showed the unusual property of increasing the permeability of chloroplast membranes to H^+ [4] in the manner of an uncoupler, but without stimulating electron flow. This effect complicates the interpretation of the mode of action of the chelators on energy-transducing functions. Therefore, other chelators were studied to determine if the H^+-permeability increase is a general property of chelators that show inhibition of phosphorylation and Mg^{2+}-ATPase functions.

METHODS

Spinach chloroplasts were prepared as previously described [8]. The light-activated and dithioerythritol-dependent Mg^{2+}-ATPase assays were conducted according to McCarty and Racker [9] with a 3 min illumination [10]. The inhibitors were added immediately after illumination so as not to interfere with the generation of the high energy state necessary for activation of the latent ATPase. Control samples were run without magnesium, and included equivalent amounts of the solvent (methanol) for the chelators. Inorganic phosphate was determined according to the method of Fiske and Subbarow [11].

The soluble, trypsin-activated, Ca^{2+}-ATPase was assayed on EDTA extracts as previously described [12]. Protein was determined according to Lowry et al. [13].

Photophosphorylation was determined as $^{32}P_i$ incorporation. $^{32}P_i$ was extracted into butanol/toluene according to Saha and Good [14] and radioactivity remaining in the aqueous phase was determined by the Cerenkov radiation-detection method of Gould et al. [15].

Electron transport rates were determined polarigraphically with a Clark-type oxygen electrode [16]. Proton translocation parameters were measured as previously described [16]. Nigericin was a generous gift of Eli Lilly and Co., Indianapolis, Indiana.

RESULTS

Electron transport, phosphorylation and ATPase

Table I shows the effect of the lipophilic chelator, BP, on whole-chain and partial electron transport. In Photosystem II electron transport, water → dimethyl benzoquinone, BP shows no inhibition. There is very little uncoupler stimulation at this site, thus it is difficult to determine the type of inhibition of BP at phosphorylation Site II. The diaminodurene → methyl viologen Photosystem I reaction appears to be stimulated slightly. Even though there is no inhibition of electron transport in either

TABLE I

EFFECT OF BATHOPHENANTHROLINE ON ELECTRON TRANSPORT

Reaction mixtures contained 100 mM KCl, 5 mM $MgCl_2$, and 20 mM Tricine · NaOH, pH 8.0 For water → methyl viologen assays, 0.5 mM methyl viologen and 0.5 mM sodium azide were added. For water → dimethyl benzoquinone assays, 0.5 mM dimethyl benzoquinone, 0.5 mM ferricyanide and 1 μM dibromothymoquinone were added. For diaminodurene → methyl viologen assays, 0.5 mM diaminodurene, 1 mM ascorbate, 5 mM DCMU, 0.5 mM methyl viologen, and 0.5 mM azide were added. Each assay contained approx. 40–60 μg chlorophyll. Concentrations of ADP and P_i were 1 mM and 3 mM respectively. The basal rate was that in the absence of ADP and P_i.

Electron trans port system	Additions	Electron transport rate (μequiv. · mg chlorophyll^{-1} · h^{-1})
Water → methyl viologen	Basal	768
	+ADP+P_i	968
	+ADP+P_i+7.5 μM BP	468
	+ADP+P_i+15 μM BP	184
	+ADP+P_i+30 μM BP	0
	+ADP+P_i+30 μM BP+ 0.025 μM gramicidin	0
Water → dimethyl benzoquinone	Basal	234
	+ADP+P_i	284
	+ADP+P_i+75 μM BP	250
	+ADP+P_i+150 μM BP	234
Diaminodurene → methyl viologen	Basal	1239
	+ADP+P_i	1342
	+ADP+P_i+150 μM BP	1170
	+ADP+P_i+600 μM BP	1305

TABLE II

EFFECT OF DPPD ON ELECTRON TRANSPORT

Chloroplasts were prepared as described in Methods. Assays were performed as described in Table I

Electron transport system	Additions	Electron transport rate (μequiv · mg chlorophyll^{-1} · h^{-1})
Water → methyl viologen	Basal	361
	+ADP+P_i	496
	+ADP+P_i+1.0 mM DPPD	361
	+ADP+P_i+1.0 mM DPPD+ 0.025 μM gramicidin	564
Water → dimethyl benzoquinone	Basal	268
	+ADP+P_i	394
	+ADP+P_i+0.5 mM DPPD	338
	+ADP+P_i+1.0 mM DPPD	310
	+ADP+P_i+1.0 mM DPPD 2.0 μM CCCP	296
Diaminodurene → methyl viologen	Basal	230
	+ADP+P_i	242
	+ADP+P_i+0.5 mM DPPD	254
	+ADP+P_i+1.0 mM DPPD	230
	+ADP+P_i+1.0 mM DPPD+ 2.0 μM CCCP	248

partial reaction, whole chain (water → methyl viologen) electron transport is severely inhibited at 15–30 μM BP, and the inhibition is not reversed by uncouplers or ionophores, as reported previously [4].

Another lipophilic chelator, DPPD, appears to behave as a classical energy-transfer inhibitor (Table II). In the water → methyl viologen system, the coupled, but not the basal electron transport is inhibited, and the electron transport inhibition is relieved by uncouplers such as CCCP and gramicidin. Again, the partial electron transfer reactions in either Photosystem I or Photosystem II are not inhibited.

Fig. 1 shows that BP inhibits phosphorylation in cyclic, whole-chain noncyclic and the Photosystems I and II phosphorylation. The Photosystem II partial reaction, water → dimethyl benzoquinone, shows less inhibition at concentrations which inhibit Site I completely. As seen in Table III, DPPD also inhibits all noncyclic modes and cyclic phosphorylation. The previous study [4] showed that TTFA, a lipophilic diketone chelator, gave 90 % inhibition of ATPase and phosphorylation activities at concentrations of 1–3 mM. The diketone chelator DPPD also inhibits the membrane-bound, light- and dithioerythritol-dependent ATPase, (about 80 % inhibition at 2 mM), but not the soluble enzyme Ca^{2+}-ATPase (data not shown). *o*-Phenanthroline (2 mM), a water-soluble chelator, gave only 27 % inhibition of the Mg^{2+}-ATPase, in agreement with the previous study [4] which showed that the water-soluble chelator, Tiron, was not inhibitory.

Effect of chelators on proton translocation

The inhibitory functions of BP and DPPD become more complex when their effect on proton translocation is examined. Both chelators completely inhibit net

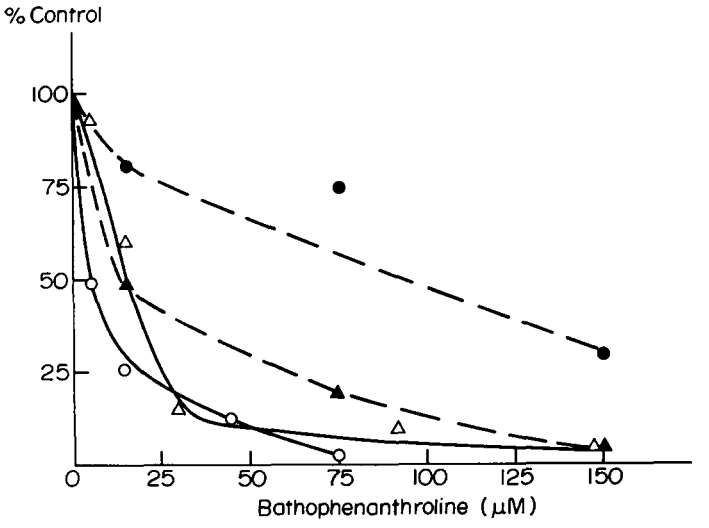

Fig. 1. Inhibition of photophosphorylation by bathophenanthroline. Chloroplasts were prepared as described in Methods. Photophosphorylation assays were conducted in 100 mM $MgCl_2$, 20 mM Tricine · NaOH, pH 8.0, 1 mM ADP, 3 mM P_i and approx. 0.4 μCi ^{32}P; per 2 ml total volume of reaction mixture. For water → dimethyl benzoquinone (Site II) phosphorylation, 0.5 mM ferricyanide, 0.5 mM dimethyl benzoquinone and 1 μM dibromothymoquinone were added. For water → methyl viologen (Site I and II), 0.5 mM methyl viologen and 0.5 mM azide were added. For diaminodurene → methyl viologen (Site I), 0.5 mM methyl viologen, 0.5 mM azide, 5 μM DCMU, 0.5 mM diaminodurene, and 1 mM ascorbate were added. For cyclic phosphorylation, 30 μM phenozine methosulfate was added. Each assay contained 40–60 μg chlorophyll. Samples were illuminated for 30 s at 15 °C with saturating light, and the reaction was stopped by freezing rapidly at —20 °C. The control rates are in μmol ATP formed mg chlorophyll^{-1} · h^{-1}. ●—●, water → dimethyl benzoquinone (control = 55), ○—○, water → methyl viologen (269); △—△, diaminodurene → methyl viologen (300); ▲—▲, phenozine methosulfate cyclic (349). [^{32}P]ATP was determined after the removal of unreacted orthophosphate as phosphomolybdic acid in butanol/toluene [14]. Specific activity of ^{32}P-labeled orthophosphate was established by counting a 0.2 ml aliquot of the phosphorylation media. All samples were counted using a Beckman DPM-100 scintillation counter with a counting error not in excess of 1 %. Typical values were approx. 40000 cpm per 0.2 ml of reaction mixture for the specific activity. Phosphorylation rates were determined by adding 1 ml aliquots from the aqueous phase of the butanol/toluene extracted samples. Typical values were 5000–7000 cpm per 1 ml aliquot of the 14.8 ml total volume aqueous phase for control samples and 200–300 cpm for samples showing nearly complete inhibition.

proton uptake and increase the k_d, indicating that they induce proton leakiness in the membrane (Table IV).

Under the conditions we used (i.e. a 15-min period for the ATPase to function), uncouplers and ionophores, at concentrations which induce a proton leak and decrease the extent of proton uptake, inhibited the Mg^{2+}-ATPase, if added during light activation (data not shown). When the inhibitors were added in the dark phase after the light phase, inhibition of the ATPase was observed with the ionophores gramicidin and nigericin, but less inhibition was seen with the uncouplers NH_4Cl and CCCP. From these results alone, we were unable to discern whether the ATPase and phosphorylation inhibition were due to induced membrane leakiness or a more specific chelator effect with DPPD and BP.

TABLE III

EFFECTS OF DPPD ON PHOTOPHOSPHORYLATION

Chloroplasts were prepared as described in Methods. Assays were conducted as described in Figure 1 Control rates, in brackets, are in μmol ATP formed · mg chlorophyll^{-1} · h^{-1}.

Electron transport system and additions	% Inhibition
$H_2O \rightarrow$ methyl viologen	
Control	0 (267)
+10 μM DPPD	20
+50 μM DPPD	73
+100 μM DPPD	80
+500 μM DPPD	98
$H_2O \rightarrow$ dimethyl benzoquinone	
Control	0 (69)
+10 μM DPPD	12
+50 μM DPPD	80
+100 μM DPPD	89
+500 μM DPPD	94
Diaminodurene $\rightarrow$ methyl viologen	
Control	0 (644)
+100 μM DPPD	57
+500 μM DPPD	87
Phenazine methosulfate-Cyclic	
Control	0 (380)
+100 μM DPPD	55
+500 μM DPPD	87

TABLE IV

EFFECTS OF BP AND DPPD ON PROTON TRANSPORT

Assays were performed with 100 mM KCl, 5 mM $MgCl_2$, 1 mM Tricine–NaOH, pH 8.0, 30 μM phenazine methosulfate, and 40–60 μg chlorophyll per 2 ml total volume. Reactions were conducted with heat-filtered saturating white light. Changes in pH of the suspending media were recorded on a Sargent Model SRG strip chart recorder. (Full scale 0.1 pH units). The buffering capacities of each reaction were determined by injection of known amounts of acid. Control experiments (no additions) typically resulted in a pH rise of approx. 0.02 pH units. The k_d is the rate constant for H^+ efflux in the dark and the extent is total uptake of protons in the steady state.

Additions	H^+ transport parameters	
	H^+ (Extent) (μmol H^+ · mg chlorophyll^{-1})	k_d (s^{-1})
None	0.105	0.09±0.02
10 μM BP	0.052	0.15±0.01
25 μM BP	0.035	0.20±0.03
50 μM BP	0.008	0.46±0.01
75 μM BP	0	–
0.05 mM DPPD	0.076	0.23±0.01
0.2 mM DPPD	0.021	0.37±0.01
0.3 mM DPPD	0	–

TABLE V

Part A. Effect of nonanedione (ND) on the Mg^{2+}-ATPase, proton gradient, phosphorylation and electron transport. Proton transport (cyclic system) assay conditions were as described in Table IV, and the phosphorylation assay as in Fig. 1. ATPase assays were conducted with 50 mM Tris · HCl, pH 8.0, 50 mM NaCl, 5 mM dithioerythrytol, 5 mM $MgCl_2$, 50 μM pyocyanine and 0.6 mg chlorophyll (chl) in a total volume of 2 ml. After a 3 min illumination, the chelator plus 5 mM ATP were added. $MgCl_2$ was omitted in control samples. After incubation for 15 min at 38 °C, the reaction was terminated with trichloroacetic acid, and the inorganic phosphate was determined according to Fiske and Subbarow [11]. The absorbance of the phosphomolybdate complex was read at 730 nm. Samples showing optimal ATPase activities typically had values of approx. 0.800–0.900 A for 0.25 ml aliquots. The $-Mg^{2+}$ or $-Ca^{2+}$ tubes had average absorbances of 0.090–0.100. These values were subtracted from the sample A and the rates were determined from a standard curve with values of 0.15, 0.3, 0.6, 1.2 μmol P_i in 3 ml. Part B. For the series B experiments (non-cyclic system), the nonanedione at 10 mM was sonicated into 100 mM KCl, 5 mM $MgCl_2$ to form a micellar dispersion. Aliquots of this micellar suspension were added to reaction media used for measuring phosphorylation and proton transport by the ΔpH method as follows: phosphorylation; 100 mM KCl, 5 mM $MgCl_2$, 3 mM K_2HPO_4, 1 mM ADP, 0.5 mM methyl viologen and 0.4 mM sodium azide, at pH 8.0 and with chloroplasts equivalent to 26 μg chlorophyll per ml. Proton transport; as above without the ADP. White light was used at saturating intensities and the temperature was 20 °C. Part C. The electron transport assays of Part C were carried out using the sonicated nonanedione procedure as outlined above, with the nonanedione being sonicated into the sucrose · tricine · $MgCl_2$ medium at 10 mM from which appropriate dilutions were prepared. The assay system consisted of the following medium: 100 mM sucrose, 50 mM tricine, pH 8.0, 3 mM $MgCl_2$, 3 mM K_2HPO_4, $\pm$1 mM ADP, 0.5 mM methyl viologen and 0.4 mM sodium azide.

Additions	% Control ATPase	Phosphorylation (μmol ATP · mg chl^{-1} · h^{-1})	H^+ Transport Parameters	
			H^+ (extent) (μmol H^+ · mg chl^{-1})	k_d (s^{-1})
A. None	100 (63.4)*	163	0.04	0.12±0.02
1.0 mM ND	44	138	–	0.09±0.01
2.0 mM ND	22	71	0.21	0.10±0.01
B. None	–	202	0.10	0.14
1.0 mM	–	121	–	–
2.0 mM	–	58	–	–
3.0 mM	–	23	0.23	0.17
5.0 mM	–	0	–	–

	Electron transport (μequiv e^- · mg chl^{-1} · h^{-1})
C. 1. Basal ($-P_i$, $-$ADP)	390
2. Coupled ($+P_i$, $+$ADP)	560
$+1.5$ μM nigericin	606
3. Coupled+1 mM ND	530
4. Coupled+2 mM ND	403
5. Coupled+3 mM ND	326
$+1.5$ μM nigericin	541

* Number in brackets is specific activity in μmol P_i released/mg chlorophyll per h.

To separate the chelator and proton-permeability effects of DPPD and BP, if indeed there were two effects, we searched for a chelator that did not induce proton leakiness. These characteristics were found in 4,6-nonanedione, a highly lipophilic diketone chelator*. As seen in Table V, the effect of 4,6-nonanedione on the rate constant of proton efflux is somewhat variable. In one series of experiments (Part A, utilizing the phenazine methosulfate cyclic system) there was only a slight increase on the rate constant for efflux, with a 5-fold increase in the extent of H^+ uptake. The other series (Part B, utilizing the methyl viologen non-cyclic system) gave a marginal increase in k_d with 3 mM 4,6-nonanedione and a two-fold increase in the extent of H^+ uptake (in other experiments the increase in the extent was close to 4-fold). Photophosphorylation measured in the same series of experiments was completely inhibited by 5 mM 4,6-nonanedione.

Nonanedione, at 2–3 mM, inhibited the coupled electron flow back to about the basal rate, and the inhibition was completely reversed by the uncoupler nigericin (Table V, part C).

DISCUSSION

The lipophilic chelators BP and DPPD exert their inhibitory effect on electron transport in the region around plastoquinone and cytochrome *f*, since they have no effect on the partial (Photosystem I or Photosystem II) electron transfer reactions (Tables I and II). The only part of the non-cyclic electron transport chain not utilized by the partial reactions is the plastoquinone-cytochrome *f* region. These chelator inhibitions are thus consistent with, but do not prove, the existence of a non-heme, iron-protein redox component between plastoquinone and cytochrome *f*. As mentioned in the Introduction, recent ESR results have suggested this possibility [5, 6]. Speculative theoretical work by Wang and Copeland [17] has led to the suggestion that a metal such as iron might function in coupling electron transport to ATP formation.

The fact that the chelators BP and DPPD inhibit phosphorylation coupled to either the Photosystems I or II partial reactions as well as the whole-chain phosphorylation but do not inhibit electron flow in the partial systems, makes the Wang and Copeland hypothesis rather tenuous. The present results seem to rule out an obligatory role of a metal acting as a redox link between electron flow and ATP formation.

However, our results are consistent with a metal acting some way in concert with the coupling factor in the energy transduction step(s). The inhibitory action of lipophilic chelators and lack of inhibition by water-soluble chelators obviously suggests that the inhibition site is buried in (or behind) a lipophilic barrier within the membrane.

The inhibitory effects of such diverse chelators as the phenanthrolines and diketones lends credence to the suggestion that a chelator-sensitive metal (such as iron) is the target of these chelators rather than the compounds exerting a non-specific, membrane-disruption effect. Along this line, it is of interest that the ATPase

* The relatively high concentrations of nonanedione used in these experiments gave a phase separation of the organic chelator from the aqueous phase. An attempt to alleviate this involved sonicating nonanedione into the aqueous phase as micelles. This resulted in a better dispersion of the chelator throughout the reaction media.

in *E. coli* shows a zinc requirement for its function [18] and Ernster [19] has reported preliminary evidence for 1 or 2 iron atoms attached to the mitochondrial F_1 coupling factor.

Nonanedione, a lipophilic diketone chelator, inhibits phosphorylation and the Mg^{2+}-ATPase, but does not induce significant membrane leakiness to H^+ (Table V), unlike BP and DPPD which greatly enhance H^+ permeability (Table IV). This result indicates that there is very probably an effect of all the lipophilic chelators studied here on energy transduction apart from the H^+ leakiness induced by BP and DPPD. The stimulation of H^+ uptake by 4,6-nonanedione is somewhat analogous to the action of such energy transfer inhibitors as D10-9 [20] and dicyclohexylcarbodiimide [21]. The mechanism whereby proton uptake is stimulated by 4,6-nonanedione remains to be determined.

The results of these studies should stimulate further work on the role of metals in energy transduction.

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REFERENCES

1 Phelps, D. C., Harmon, H. J. and Crane, F. L. (1974) Biochem. Biophys. Res. Commun. 59, 1185
2 Sun, I. L., Phelps, D. C. and Crane, F. L. (1975) FEBS Lett. 54, 253
3 Barr, R. and Crane, F. L. (1974) Biochem. Biophys. Res. Commun. 60, 748
4 Bering, C. L., Dilley, R. A. and Crane, F. L. (1975) Biochem. Biophys. Res. Commun. 63, 736
5 Malkin, R. and Aparicio, P. J. (1975) Biochem. Biophys. Res. Commun. 63, 1157
6 Prince, R. C., Lindsay, J. G. and Dutton, P. L. (1975) FEBS Lett. 51, 108
7 Rieske, J. S., Hansen, R. E. and Zuagg, W. S. (1964) J. Biol. Chem. 239, 3017
8 Dilley, R. A., Peters, G. A. and Shaw, E. R. (1972) J. Membrane Biol. 8, 163
9 McCarty, R. E. and Racker, E. (1968) J. Biol. Chem. 243, 129
10 Rienits, K. G. (1967) Biochim. Biophys. Acta 143, 595
11 Fiske, C. H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375
12 Giaquinta, R. T., Selman, B. R., Bering, C. L. and Dilley, R. A. (1974) J. Biol. Chem. 249, 2873
13 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265
14 Saha, S. and Good, N. E. (1970) J. Biol. Chem. 245, 5017
15 Gould, J. M., Cather, R., Winget, G. D. (1972) Anal. Biochem. 50, 540
16 Dilley, R. A. (1970) Arch. Biochem. Biophys. 137, 270
17 Wang, J. H. and Copeland, L. (1974) Arch. Biochem. Biophys. 162, 64
18 Sun, I. L. and Crane, F. L. (1975) Biochem. Biophys. Res. Commun. 65, 1334
19 Ernster, L. (1975) 10th FEBS Symposium, North Holland Press, Amsterdam
20 Karlish, S. J. D. and Avron, M. (1968) Biochim. Biophys. Acta 153, 878
21 McCarty, R. E. and Racker, E. (1967) J. Biol. Chem. 242, 3435