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ARGININE MODIFIERS AS ENERGY TRANSFER INHIBITORS IN PHOTOPHOSPHORYLATION

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SUMMARY

Photophosphorylation by spinach chloroplasts is inhibited after they have been incubated in the dark with either phenylglyoxal or butanedione. Inhibition by phenylglyoxal is strongest when *N*-ethylmorpholine is the buffer used during the incubation; that by butanedione requires the presence of borate as buffer. The inhibitions are not reversed by simply washing out the inhibitor, suggesting that a covalent modification of one or more arginine residues is responsible. This is supported by the reversibility of the butanedione inhibition if both the inhibitor and borate buffer are removed. ATPase of the chloroplasts, and of extracted protein, is inhibited, whether activated by trypsin or by heating. This indicates that arginine residues of the coupling factor are the probable major site(s) for attack by these modifiers, leading to the observed inhibitions.

INTRODUCTION

Covalent chemical modifiers can be of use in defining aspects of enzyme function, whether they act at the reaction center, affect ligand binding, or change protein conformational relations. Studies with the coupling factor (CF_1) of spinach chloroplasts still attached to the green membranes indicated reactivity of presumed sulfhydryl group(s) to covalent modifiers such as *N*-ethylmaleimide [1], *o*-iodosobenzoic acid [2], bis-dithionitropyridine [3], or copper ions (Uribe, E., personal communication) only when the membranes were in the high energy state. Sensitivity to oxidation by permanganate, also presumably on the part of a sulfhydryl group, was accelerated by the high energy state [4]. Similarly up to four amino groups of CF_1 were modified by trinitrobenzene sulfonate, leading to inhibition of the enzyme's ATPase function, when previously amidinated chloroplasts were exposed to the inhibitor and illuminated at the same time [5]. With the enzyme detached from the membranes, covalent binding

Abbreviations: CF_1, the coupling factor of chloroplasts; F_1, the coupling factor of mitochondria; NBD-chloride, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; Tris, tris(hydroxymethyl)aminomethane; Tricine, *N*-tris(hydroxymethyl)methyl glycine; MOPS, morpholinopropane sulfonic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid; DCPIP, dichlorophenolindophenol.

of NBD-chloride to one tyrosine on the B subunit caused inhibition of its ATPase activity [6].

Within recent years glyoxal-type compounds such as butanedione, phenylglyoxal and cyclohexanedione [7–20] have been used as reagents able to form covalent complexes specifically with arginine in proteins. The stability of any complex is usually highly dependent on the nature of the buffer. Butanedione, for instance, is easily lost unless borate is present [9–14, 18]. This characteristic makes the reagents useful for reversible modifications, increasing the correlations between binding of the specific group and the degree of inhibition or other alteration of enzymatic behavior. Most recently butanedione and phenylglyoxal have been used to indicate the probable function of an arginine residue at the active site of solubilized F_1 from rat liver and beef heart mitochondria [20].

In this study we have used arginine-specific reagents on whole chloroplasts. Under carefully defined conditions they behave as specific energy transfer inhibitors, acting on photophosphorylation and on the ATPase activity of CF_1 without interfering with either basal electron flow rates or proton pumping.

MATERIALS AND METHODS

Leaves from greenhouse-grown spinach plants were homogenized in 0.4 M sorbitol, 20 mM Tricine at pH 7.8, and 10 mM NaCl. Chloroplasts were collected by centrifuging at $7000 \times g$ for 10 min, and washed once in 10 mM NaCl. For experiments with phenylglyoxal they were resuspended in 0.4 M sorbitol and 10 mM NaCl at 1 mg chlorophyll per ml. For experiments with butanedione they were resuspended in 20 mM borate, pH 7.9 (concentration listed is that of boric acid), 50 mM choline chloride, 1 mM dithiothreitol, and 0.2 mg/ml bovine serum albumin. In the text this is referred to as the standard borate buffer.

Recrystallized phenylglyoxal hydrate, with a melting point of 92 °C, was dissolved in 125 mM *N*-ethylmorpholine chloride at pH 7.8. In the reaction with phenylglyoxal the incubation mixture contained chlorophyll with 0.33 mg/ml of chlorophyll, 80 mM *N*-ethylmorpholine chloride at pH 7.8, 140 mM sorbitol, 5 mM NaCl, 1 mM dithiothreitol, 0.2 mg/ml bovine serum albumin and varying amounts of phenylglyoxal as indicated. Incubation was for 20 min at 20 °C; then the mixture was diluted with seven volumes of 0.40 M sorbitol, 10 mM NaCl, the chloroplasts centrifuged, resuspended in half the original volume of sorbitol/NaCl, and washed once more. (The extra wash step was necessary to remove all *N*-ethylmorpholine, which seems to inhibit photophosphorylation.) The final chlorophyll concentration was 0.5 mg/ml in sorbitol/NaCl.

Butanedione was mixed with 100 mM borate buffer at pH 7.9. When chloroplasts were reacted with butanedione the mixture contained 0.25 mg/ml of chlorophyll, 55 mM borate at pH 7.9, 50 mM choline chloride, 1 mM dithiothreitol and 0.2 mg/ml of bovine serum albumin. The incubation was for 20 min at 20 °C, after which 10 volumes of cold standard borate buffer were added, the chloroplasts centrifuged down and resuspended to 0.5 mg chlorophyll per ml in fresh standard buffer.

To extract CF_1 chloroplasts were suspended at 0.06 mg chlorophyll per ml in 0.4 mM sorbitol and 0.75 mM EDTA at pH 7.8 for 15 min at room temperature, then centrifuged.

ATPase of chloroplasts was activated using trypsin at 0.25 mg/ml [21] and assayed by procedures described earlier [4]. ATPase of the soluble extract from chloroplasts was activated using 0.05 mg trypsin per ml. Heat activation was as described by Lien and Racker [22]. In all cases the released inorganic phosphate was determined colorimetrically [23]. Photophosphorylation was measured as described earlier [24] with unreacted radioactive $^{32}P_i$ removed by precipitating the phosphomolybdate complex with triethylamine [25].

Soluble proteins were measured by the Lowry et al. procedure [26] after precipitation with trichloroacetic acid in the presence of deoxycholate [27].

RESULTS

Incubation of spinach chloroplasts for 20 min at a temperature of 20 °C was found to be optimum for demonstrating the inhibition by either phenylglyoxal or butanedione, without causing too much damage to the control chloroplasts incubated without these reagents. Even after removing excess reagent by two successive washes, photophosphorylation was almost completely inhibited (Table I).

TABLE I

EFFECT OF PHENYLGLYOXAL AND OF BUTANEDIONE ON PHOSPHORYLATION AND ELECTRON TRANSPORT

Chloroplasts with a final chlorophyll concentration of 0.33 mg/ml for *N*-ethylmorpholine or 0.25 mg/ml for butanedione were incubated and washed as described under Materials and Methods. Electron transport was measured as O_2 uptake using a Clark type electrode in a medium consisting of 0.1 M sorbitol, 50 mM Tricine at pH 8, 25 mM NaCl, 5 mM $MgCl_2$, 50 μM methyl viologen and 2.5 mM NaN_3. Where indicated, final concentrations were 3 mM ADP, 3 mM P_i, 2 mM NH_4Cl, 0.75 mM ATP, 60 μM DCPIP and 2.3 mM ascorbate. In experiments with butanedione 3 mM borate and 7 mM choline chloride were also present. Rates are shown as μequiv./mg chlorophyll per h. Photophosphorylation was assayed as described under Materials and Methods. With butanedione 2 mM borate and 5 mM choline chloride were also present during the assay. The photophosphorylation rate of chloroplasts not given a 20 min preincubation at 20 °C was 797 μmol/mg chlorophyll per h.

Reaction	Pretreatment by incubation in			
	N-Ethylmorpholine · HCl		Borate	
	–	+19.2 mM phenylglyoxal	–	+20 mM butanedione
Phosphorylation	598	30	533	106
Electron transport				
H_2O to methyl viologen	253	293	265	265
+ADP/P	375	267	374	219
+NH_4Cl	609	–*	567	575
+ATP	232	245	249	227
DCPIP/ascorbate to methyl viologen	392	440	324	390
+NH_4Cl	540	608	590	648

* In this reaction oxygen uptake showed an unimpaired initial rate, then declined to zero during 3 min of illumination.

As with other enzymes [9–15, 18, 20], borate buffer had to be present for butanedione to inhibit. With phenylglyoxal the nature of the buffer was also critical. In this case, however, *N*-ethylmorpholine permitted the strongest inhibition to occur. Tris, MOPS or borate buffers were less effective, and almost no inhibition by phenylglyoxal occurred when using either Tricine or HEPES buffers (data not shown).

Inhibition occurred during the incubation of chloroplasts with phenylglyoxal or butanedione in darkness. No greater inhibition due to light or the high energy state has been found as yet. Experiments to test this possibility were performed with MOPS buffer, as *N*-ethylmorpholine uncouples electron transport and inhibits photophosphorylation under our conditions (unpublished observations).

The nature of inhibition by phenylglyoxal and butanedione is explored in Tables I and II. The data fit the pattern for a simple energy transfer inhibitor most clearly with butanedione. The basal rate of electron transport was not affected even when photophosphorylation was inhibited 78 % (Table I, columns 3 and 4). Stimulation of electron flow by ADP and P_i was eliminated by butanedione pretreatment; but the stimulation by an uncoupler (NH_4Cl) was unaffected. The uncoupler effect could be observed whether electron transport was through Photosystem I only (bottom 2 lines) or through both Photosystems I and II.

With phenylglyoxal-treated chloroplasts the same pattern was evident except for electron flow through both photosystems when NH_4Cl as uncoupler was present. Basal electron flow was not affected (Table I), and NH_4Cl stimulation occurred in electron flow through Photosystem I only. However, in the presence of NH_4Cl electron

TABLE II

INHIBITION OF PHOSPHORYLATION AND OF PHOTOSYSTEM II BY PHENYLGLYOXAL WITH MORE LABILE CHLOROPLASTS

Chloroplasts were preincubated in 80 mM *N*-ethylmorpholine chloride and 5.5 mM NaCl, with or without phenylglyoxal, as in Table I, except that dithiothreitol and bovine serum albumin were omitted. Control chloroplasts were stored in 0.4 M sorbitol and 10 mM NaCl at 0 °C during this time. Phosphorylation and electron transport were measured as in Table I and Materials and Methods. For phosphorylation and proton uptake 40 μM methyl phenazinium chloride and 12 μM Diuron were present to make sure only Photosystem I was operating. Proton uptake was measured with a glass electrode, calibrated by HCl titration. Chloroplasts were resuspended in 25 mM NaCl and used at 0.04 mg chlorophyll per ml with an initial pH between 6 and 6.5. Electron transport rates are shown as μequiv./mg chlorophyll per h; phosphorylation rates as μmol ATP/mg chlorophyll per h; and proton uptake as μequiv. H^+/mg chlorophyll in the steady state.

Reaction	Control	Preincubated, 20 min at 20 °C	
		–	Phenylglyoxal
Phosphorylation	740	268	14
Proton uptake	0.65	0.27	0.25
Electron transport:			
H_2O to methyl viologen	196	229	81
$+NH_4Cl$	504	316	81
DCPIP/ascorbate to methyl viologen	480	461	508
$+NH_4Cl$	693	746	712

transport from H_2O as donor was not stable in the treated chloroplasts and a progressive loss in activity occurred during the reaction until no electron transport remained by 3 min.

A further complication comes from our observation that different batches of chloroplasts varied in their susceptibility to phenylglyoxal inhibition of Photosystem II electron transport. With some (as in Table I) the basal rate was not affected, but progressive inhibition occurred in the presence of NH_4Cl. With others Photosystem II basal electron transport (measured from initial rates) was inhibited strongly following pretreatment with phenylglyoxal (Table II). In these, adding NH_4Cl neither stimulated the initial rate nor caused the progressive decline of activity with time in the light.

The pronounced inhibition of Photosystem II basal electron transport by phenylglyoxal seemed to be correlated with a greater lability of these chloroplasts to the preincubation conditions, even without arginine modifiers. Note that the chloroplasts of Table II had lost 64 % of their photophosphorylation rate due to the 20 min incubation at 20 °C and subsequent washings; whereas those of Table I had lost only 25 % from the same cause.

The extent of inhibition as a function of the phenylglyoxal concentration is shown in Fig. 1, and as a function of butanedione concentration in Fig. 2. Relative linearity of inhibition with the log of the inhibitor concentration is apparent, especially for butanedione. Simultaneous measurements were made of trypsin-activated ATPase [21]. It is apparent that the inhibitions of ATPase and of photophosphorylation are closely correlated.

Low concentrations of the arginine-binding reagents stimulated photophosphorylation but not ATPase (Figs. 1 and 2). If the stimulation results from partial modification of the same sites as those whose complete modification causes inhibition, we would expect to see a transitory rise in ATP synthesis rates during the early moments of exposure of chloroplasts to higher concentrations of the inhibitors. However, the time-course studies showed only a monotonic, pseudo first-order reaction leading

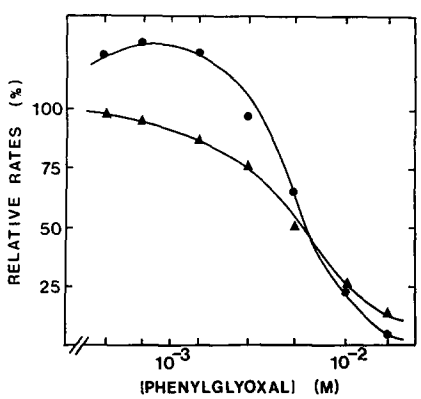

Fig. 1. Dependence of the phosphorylation rate (● — ●) and ATPase activity (▲ — ▲) of chloroplasts on the concentration of phenylglyoxal during incubation. Incubation conditions and assays were carried out as described under Materials and Methods. The phosphorylation rate of the control (100 %) was 392 μmol ATP/mg chlorophyll per h and the ATPase control rate was 583 μmol P_i released/mg chlorophyll per h.

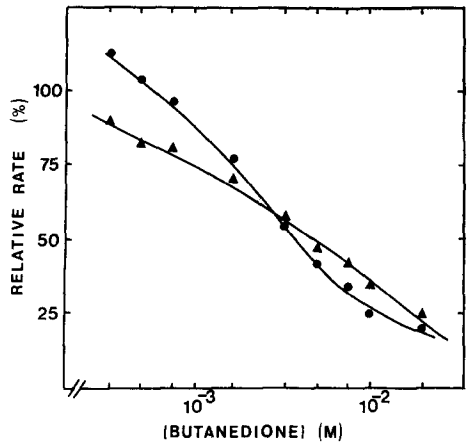

Fig. 2. Dependence of the phosphorylation rate (● — ●) and ATPase activity (▲ — ▲) of chloroplasts on the concentration of butanedione during incubation. Incubation conditions and assays as under Materials and Methods. The control phosphorylation rate (= 100 %) was 324 μmol ATP/mg chlorophyll per h and the control ATPase rate was 463 μmol P_i/mg chlorophyll per h.

to inhibition of ATP synthesis at all inhibitory levels of phenylglyoxal (Fig. 3) with no indication of a preliminary stimulation or even lag. Hence the stimulatory effect of a 20 min exposure to low concentrations probably results from binding at a site other than the one on CF_1 leading to inhibition. This inferred second site would be charac-

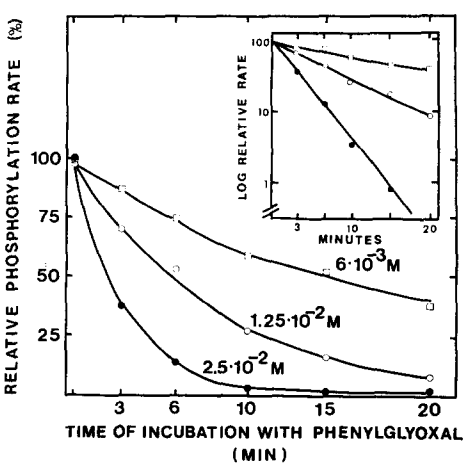

Fig. 3. Dependence of the phosphorylation rate of chloroplasts on the time of incubation with different concentrations of phenylglyoxal. Chloroplasts were incubated with phenylglyoxal as described under Materials and Methods, and aliquots were removed at the indicated times. They were immediately diluted, centrifuged, and washed with 0.4 M sorbitol and 10 mM NaCl. Control chloroplasts were incubated under the same conditions but without phenylglyoxal, and aliquots removed at the same time as the treated samples. Rates of the phenylglyoxal-treated chloroplasts are shown as percent of the rate of these control chloroplasts. Control phosphorylation rates decreased from 430 μmol ATP/mg chlorophyll per h at time 0, to 365 μmol ATP/mg chlorophyll per h after 20 min. The insert shows a semilogarithmic plot of the time-courses for the phenylglyoxal effect.

terized by sensitivity to lower concentrations of phenylglyoxal or butanedione than those needed to inhibit photophosphorylation. Low concentrations of phenylglyoxal did not stimulate proton uptake after removal of CF_1 by EDTA, hence, this site is probably not the same one as that to which dicyclohexyl carbodiimide binds.

We should note, in addition, that the stimulation by low concentrations was somewhat variable. While it always was found with chloroplasts whose intrinsic activity was low, it was usually (but not always) absent from those with photophosphorylation rates on the order of 500 μmol/mg chlorophyll per h or better. More work is needed to obtain an understanding of this stimulation.

For purposes of further analysis it would be especially useful if the observed inhibitions were caused by covalent modifications of CF_1. Any such modifications would remain with the enzyme after it is detached from the chloroplast membranes, and show up as an inhibition of the ATPase function of the soluble enzyme. Evidence that a modified, inhibited CF_1 can still be extracted by EDTA at low ionic strength is shown in Table III. First, just as much protein appears in the supernatant from extracted modified chloroplasts as from control chloroplasts. Second, just as with control chloroplasts extracted by EDTA, the residual membranes from modified chloroplasts show a much diminished ability to take up protons in the light, and this activity is restored by addition of dicyclohexyl carbodiimide [28]. Third, gel electrophoresis of the concentrated EDTA extract showed a protein band in the same position as that of CF_1 in the extract from control chloroplasts. Also, electrophoresis of the supernatant in the presence of sodium dodecyl sulfate demonstrated the presence of the normal five subunits of CF_1.

In spite of the appearance of presumptive CF_1 protein in the supernatant from phenylglyoxal-treated chloroplasts, no ATPase activity was detectable (Table III). Of even more significance was the failure to detect ATP hydrolysis after attempted activation by heating [22] in the presence of dithiothreitol. Thus inhibition of ATPase was

TABLE III

EDTA EXTRACTION OF PHENYLGLYOXAL-TREATED CHLOROPLASTS

Incubation with or without 19 mM phenylglyoxal, EDTA extraction measurements of protein concentration and ATPase rates were performed as under Materials and Methods. Proton uptake was measured as in Table II except that 25 μM pyocyanin was the electron transport mediator. Proton uptake is shown as μequiv. H^+/mg chlorophyll in the steady state; protein as mg extracted per mg chlorophyll; and ATPase as μmol P_i released/mg chlorophyll per h.

Reaction	Preincubated with	
	–	Phenylglyoxal
Proton uptake	0.48	0.45
Proton uptake after EDTA extraction	0.01	0.05
+dicyclohexylcarbodiimide	0.17	0.19
Protein in supernatant	1.25	1.25
ATPase in supernatant:		
Activated by trypsin	498	31
Activated by heat	574	50

not caused by a failure of the activation by trypsin, which splits only unmodified arginine or lysine-containing peptide bonds.

Arginine modifications are often reversed by removal of the buffer component that stabilizes the complex [9–14, 18, 20]. While this did not occur with phenylglyoxal, the butanedione inhibition of photophosphorylation was largely reversed by washing and resuspending the chloroplasts in Tricine rather than borate buffer. When resuspended in borate buffer, phosphorylation rates were 70 for butanedione-treated chloroplasts compared to 266 for the controls, or an inhibition of 73 %. When resuspended in Tricine buffer the control chloroplast rate of 384 was lowered to 287 due to butanedione pretreatment, or an inhibition of only 25 %.

DISCUSSION

Unlike many of the previous modifications of CF_1 [1–4] there was no sign in the present work that inhibition of photophosphorylation by phenylglyoxal or butanedione required, or was even accelerated by, the chloroplast high energy state. Either the target for these reagents is well exposed in the dark, or as they are not highly charged they may be able to penetrate into more hydrophobic regions of proteins or membranes that were not available to the other reagents in the dark.

Considering that phenylglyoxal and butanedione should be able to modify many arginine residues on chloroplast membrane proteins, the degree of specificity of their action is surprising. Butanedione's only effect, in these experiments, was that of an energy transfer inhibitor, similar to phloridzin [29] or DIO-9 [30]. The pattern includes no effect on either basal or uncoupled electron transport, but an inhibition of both photophosphorylation and the accelerated electron transport rates that go with it. Phenylglyoxal treatment produced the same pattern, including no inhibition of electron flow through Photosystem I whether an uncoupler was present or not; but deviated in causing an additional inhibition of electron transport through the Photosystem II region (Tables I and II). The nature of the inhibition of Photosystem II varied with the state of the chloroplasts; with the healthier ones the initial velocity was not affected but rates declined during the reaction, and with chloroplasts showing signs of aging a pronounced inhibition was apparent in the initial velocity. The latter observation is consistent with the concept of Auslander and Junge [31] in which Photosystem II in healthy chloroplast membranes is at least partly shielded by a barrier, probably protein, that also slows the rate of appearance of protons in the medium. In aged or mistreated chloroplasts indications were [31] that the barrier had been removed; and these may be the membranes whose Photosystem II components are more fully exposed to phenylglyoxal. However, the fact that phenylglyoxal treatment may lead to inhibition of electron flow through the Photosystem II region does not detract from the remaining evidence showing it to act as an energy transfer inhibitor for photophosphorylation.

Our current working hypothesis is that the inhibition of photophosphorylation and of ATPase is caused by the covalent chemical modification of one or more arginine residues of CF_1. While very high concentrations of phenylglyoxal may modify lysine residues [8] it is generally considered that under conditions such as those used here only arginines will be affected [11–14, 17]. The fact that inhibition by butanedione was reversed when chloroplasts were washed free of borate buffer can be considered as

especially good preliminary evidence for the essentiality of an arginine function. The rather slow time courses for onset of inhibition (Figs. 1 and 2) combined with the failure to reverse the inhibition by simply washing out the reagent, argue strongly for a covalent modification as the basis for inhibition. Since heat activation failed to demonstrate ATPase in the solubilized CF_1 protein from treated chloroplasts, a direct effect on catalytic activity is indicated, rather than preventing activation by blocking trypsin action. The completely normal appearance on gel electrophoresis of protein corresponding to CF_1 suggests that no major structural alterations occurred. Thus the inhibition is most probably analogous to that of mitochondrial F_1 by these same arginine-modifying reagents [20]. Applied to the solubilized enzyme in that case, phenylglyoxal and butanedione inhibited ATPase activity. From the kinetics of inactivation Marcus et al. [20] concluded that the modification of only one arginine at the active site was probably responsible for loss of enzymatic activity.

Further work is planned to test the concept of a covalent binding of phenylglyoxal to CF_1, determine the site of this binding if it occurs, and find out whether loss of ATPase and of photophosphorylation are due to modification of the same arginine residue(s).

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Butanedione inhibition of soluble, trypsin-activated ATPase of CF_1 was reported [32] while this paper was in press.

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REFERENCES

1 McCarty, R. E. and Fagan, J. (1973) Biochemistry 12, 1503–1507
2 Vallejos, R. H. and Andreo, C. S. (1976) FEBS Lett. 61, 95–99
3 Andreo, C. S. and Vallejos, R. H. (1976) Biochim. Biophys. Acta 423, 590–601
4 Datta, D. B., Jagendorf, A. T. and Ryrie, I. J. (1974) J. Biol. Chem. 249, 4404–4411
5 Oliver, D. and Jagendorf, A. T. (1976) J. Biol. Chem. 251, 7168–7175
6 Deters, D. W., Racker, E., Nelson, N. and Nelson, H. (1975) J. Biol. Chem. 250, 1041–1047
7 Yankeelov, J. A. (1970) Biochemistry 9, 2433–2439
8 Takahashi, K. (1968) J. Biol. Chem. 243, 6171–6179
9 Riordan, J. F. (1973) Biochemistry 12, 3915–3923
10 Young, P. C. and Schwert, G. W. (1972) Biochemistry 11, 2218–2224
11 Lange, L. G., Riordan, J. F. and Vallee, B. L. (1974) Biochemistry 12, 4361–4370
12 Daeman, F. J. M. and Riordan, J. F. (1974) Biochemistry 13, 2865–2871
13 Forster, M. and Harrison, J. H. (1974) Biochem. Biophys. Res. Commun. 58, 263–267
14 Borders, C. L. and Riordan, J. F. (1975) Biochemistry 14, 4699–4704
15 Marcus, F. (1975) Biochemistry 14, 3916–3921
16 Patthy, L. and Smith, E. L. (1975) J. Biol. Chem. 250, 565–569
17 Powers, S. G. and Riordan, J. F. (1975) Proc. Natl. Acad. Sci. U.S. 72, 2616–2620
18 David, M., Rusched, I. and Sund, H. (1976) FEBS Lett. 62, 288–292
19 Hjelmgren, T., Strid, L. and Arvidson, L. (1976) FEBS Lett. 68, 137–140

20 Marcus, F., Schuster, S. and Lardy, H. A. (1976) J. Biol. Chem. 251, 1775–1780
21 Vambutas, V. K. and Racker, E. (1965) J. Biol. Chem. 240, 2660–2667
22 Lien, S. and Racker, E. (1971) Methods Enzymol. 23, 547–555
23 Taussky, H. H. and Shorr, E. (1953) J. Biol. Chem. 202, 675–685
24 Grebanier, A. E. and Jagendorf, A. T. (1977) Biochim. Biophys. Acta 459, 1–9
25 Sugino, Y. and Miyoshi, Y. (1964) J. Biol. Chem. 239, 2360–2364
26 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
27 Bensadoun, A. and Weinstein, D. (1976) Anal. Biochem. 70, 241–250
28 McCarty, R. E. and Racker, E. (1966) Brookhaven Symp. Biol. 19, 202–212
29 Izawa, S., Connolly, T. N., Winget, G. D. and Good, N. E. (1966) Brookhaven Symp. Biol. 19, 169–188
30 McCarty, R. E., Guillory, R. J. and Racker, E. (1965) J. Biol. Chem. 240, PC 4822
31 Auslander, W. and Junge, W. (1974) Biochim. Biophys. Acta 357, 285–298
32 Adreo, C. S. and Vallejos, R. H. (1977) FEBS Lett. 78, 207–210