Biochimica et Biophysica Acta 851 (1986) 23–28
Elsevier

BBA 42085

The interaction of ferredoxin with chloroplast ferredoxin-linked enzymes

Masakazu Hirasawa [a], J. Milton Boyer [a], Kevin A. Gray [a], Danny J. Davis [b] and David B. Knaff [a,*]

[a] *Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409 and* [b] *Department of Chemistry, University of Arkansas, Fayetteville, AR 72701 (U.S.A.)*

(Received January 17th, 1986)

Key words: Ferredoxin; Nitrite reductase; Glutamate synthase; Chemical modification; Ferredoxin-enzyme interaction; (Spinach chloroplast)

As has previously been demonstrated for ferredoxin/$NADP^+$ oxidoreductase ($NADP^+$ reductase) and ferredoxin/nitrite oxidoreductase (nitrite reductase), ferrodoxin-dependent glutamate synthase has been shown to form a complex with ferredoxin. The complex (K_d = 14.5 μM) is stabilized by electrostatic forces and produces changes in the chromophore(s) of at least one of the proteins. Chemical modification of carboxyl groups on ferredoxin with glycine ethyl ester in the presence of a water-soluble carbodiimide affected the binding of ferredoxin to both glutamate synthase and nitrite reductase and also inhibited the ability of reduced ferredoxin to serve as an electron donor to both enzymes.

Introduction

Ferredoxin functions as the acceptor of electrons from Photosystem I in all organisms that carry out oxygenic photosynthesis [1]. Reduced ferredoxin serves, in turn, as an electron donor for a variety of processes, including the reduction of $NADP^+$ [2], nitrite [3,4], sulfite [5,6], thioredoxin and regulatory enzymes of the Calvin–Benson cyclic [7] and for the reductive conversion of 2-oxoglutarate plus glutamine to glutamate [8,9]. Evidence exists that two of these ferredoxin-dependent reductions, the reduction of $NADP^+$ catalyzed by ferredoxin/$NADP^+$ oxidoreductase (EC 1.18.1.2, hereafter referred to as $NADP^+$ reductase) and the reduction of nitrite to ammonia catalyzed by ferredoxin/nitrite oxidoreductase (EC 1.7.7.1, hereafter referred to as nitrite reductase), involve complexes between ferredoxin and the enzyme [10–16]. Glutamate synthase (EC 1.4.7.1, the enzyme responsible for the ferredoxin-dependent conversion of glutamine plus 2-oxoglutarate to glutamate in chloroplasts [8,9]), like $NADP^+$ [14,17] and nitrite reductases [4,18], can be purified using an affinity column in which ferredoxin is covalently attached to a Sepharose-4B matrix [9]. This suggested that glutamate synthase, like the other two chloroplast enzymes, can form a complex with ferredoxin. Evidence for such a complex is presented below.

Evidence from a number of laboratories indicated that electrostatic forces hold the complexes between ferredoxin and both $NADP^+$ reductase [10–12,19–21] and nitrite reductase [15,16] together. Recent ^{13}C-NMR investigations have implicated carboxyl side chains from three glutamate residues on *Anabaena variabilis* ferredoxin in complex formation with $NADP^+$ reductase [22]. Cross-linking studies between $NADP^+$ reductase and spinach ferredoxin have also implicated ferredoxin carboxyl groups in the

* To whom correspondence should be addressed.

Abbreviations: EDC, 1-methyl-3-(3-dimethylaminopropyl)carbodiimide; PMSF, phenylmethylsulfonylfluoride; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol.

0005-2728/86/$03.50 © 1986 Elsevier Science Publishers B.V. (Biomedical Division)

interaction with $NADP^+$ reductase [23]. Chemical modification of 3–4 carboxyl groups on ferredoxin by treatment with glycine ethyl ester in the presence of the water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), greatly inhibited the ability of ferredoxin to form a complex with $NADP^+$ reductase without affecting the protein's Fe_2S_2 center or its ability to be photoreduced by Photosystem I [24]. Chemical modification, presumably involving carboxyl groups, of ferredoxin by EDC [16] was also shown to affect the interaction of ferredoxin with $NADP^+$ reductase and with a modified form [15,25,26] of nitrite reductase. It thus was of interest to investigate the effects of carboxyl group modification on ferredoxin's ability to interact with the native form [4,15,25,26] of nitrite reductase and with glutamate synthase.

Experimental procedures

Materials

Spinach (field-grown during 1985 growing season) was used as the starting material for preparation of all proteins used in this study. Spinach ferredoxin ($A_{422\,nm}$: $A_{277\,nm} = 0.45$) was prepared according to the method of Tagawa and Arnon [27] and stored in 30 mM Tris-HCl buffer (pH 8.0) at liquid N_2 temperature until used. Spinach ferredoxin was modified by treatment with glycine ethyl ester in the presence of EDC according to the method of Vieira and Davis [24] and stored as described for native ferredoxin. The procedure used for the preparation of ferredoxin-linked glutamate synthase was essentially the same as described previously [9] except that 100 mM potassium phosphate buffer (pH 7.5) containing 200 mM NaCl, 25 mM 2-mercaptoethanol, 2 mM 2-oxoglutarate and 1 mM phenylmethylsulfonyl fluoride (PMSF), dissolved in isopropanol, replaced the isolation buffer used previously. After the acetone fractionation, dialysis was performed for 48 h using Spectrapor $M_r = 6000–8000$ cut-off dialysis tubing. The enzyme used in these experiments has an $A_{438\,nm}/A_{279\,nm}$ ratio of 0.20 and a specific activity 99 units/mg protein. Ferredoxin-linked (native) nitrite reductase [15] and $NADP^+$ reductase [17] were prepared as described previously.

Analytical methods

Absorbance spectra were measured at 4°C using a Perkin-Elmer Lambda 5 spectrophotometer with a noise level of less than $2 \cdot 10^{-4}$ A. Difference spectra were obtained using 1-cm optical pathlength split cell as described previously [28]. Glutamate synthase [9], ferredoxin-dependent nitrite reductase [25] and $NADP^+$ reductase [29,30] were assayed as described previously. Protein concentration was estimated by the method of Bradford [21] using bovine serum albumin as a standard. Dissociation constants (K_d) and apparent Michaelis constants (K_m^{app}) were calculated from plots of ΔA or V vs. [ferredoxin] using a non-linear least-squares program [32] run on an N.E.C. PC-8801 computer.

Results

Spinach ferredoxin, because of its large net negative charge [1,33] binds tightly to anion exchange resins such as DEAE-cellulose [27]. For example spinach ferredoxin cannot be eluted from a Whatman DE-52 DEAE-cellulose column by 10 mM Tris-HCl buffer (pH 8.0) containing 200 mM NaCl. However, we have found that addition of either $NADP^+$ reductase, nitrite reductase or glutamate synthase to a ferredoxin-saturated DE-52 column caused ferredoxin to be co-eluted with the enzymes when 10 mM Tris-HCl buffer (pH 8.0) containing 80 mM NaCl was applied to the column. This observation, along with the earlier demonstration that all three enzymes bind to a ferredoxin-Sepharose 4B affinity column at low ionic strength and elute from the affinity column as the ionic strength is raised [4,9,17,18], suggest that glutamate synthase resembles nitrite and $NADP^+$ reductases in forming an electrostatic complex with ferredoxin.

Additional evidence for complex formation between ferredoxin and the three chloroplast ferredoxin-dependent enzymes comes from the observation that ferredoxin co-migrates with each of the enzymes during gel filtration chromatography. Fig. 1 shows that, as has been previously demonstrated [34], ferredoxin and $NADP^+$ reductase co-migrate during chromatography on Sephadex G-75 at low ionic strength with an apparent M_r for the complex of 48 kDa (determined according to

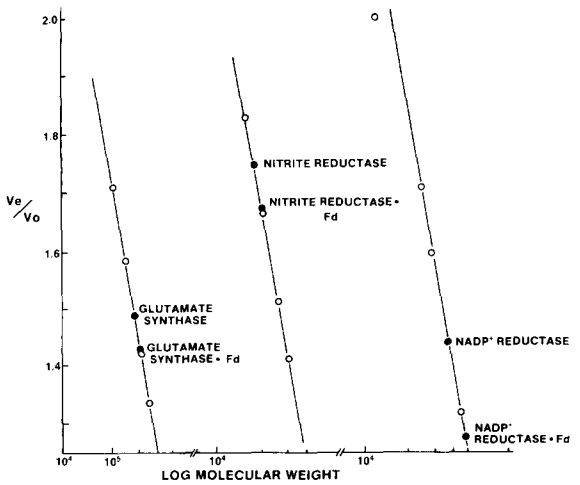

Fig. 1. Gel filtration chromatography of enzyme·ferredoxin complexes. (A) Glutamate synthase. Chromatography was performed on an Ultrogel AcA 34 column (1 × 30 cm) equilibrated with 10 mM potassium phosphate buffer (pH 7.5) containing 12.5 mM 2-mercaptoethanol and 2 mM 2-oxoglutarate. Molecular weight standards (○) used: hexokinase, 110 kDa; alcohol dehydrogenase, 150 kDa; β-amylase, 200 kDa and catalase, 240 kDa. (B) Nitrite reductase. Chromatography was performed on an Ultrogel AcA 34 column (1 × 30 cm) equilibrated with 30 mM Tris-HCl buffer (pH 8.0). Molecular weight standards (○) used: bovine serum albumin, 68 kDa; hexokinase, 110 kDa; alcohol dehydrogenase, 150 kDa and β-amylase, 200 kDa. (C) $NADP^+$ reductase. Chromatography was performed on a Sephadex G-75 column (1 × 25 cm) equilibrated with 30 mM Tris-HCl Buffer (pH 8.0). Molecular weight standards (○) used: equine cytochrome *c*, 12.4 kDa; trypsinogen, 24 kDa; carbonic anhydrase, 29 kDa and ovalbumin, 45 kDa.

Andrews [35]). If the chromatography on G-75 were repeated under conditions where the electrostatic complex would be expected to dissociate (in buffer supplemented with 200 mM NaCl to raise the ionic strength), no co-migration was observed and the ferredoxin and $NADP^+$ reductase eluted as separate components with elution volumes expected from their respective molecular weights of 10 500 [33] and 36 000 [36–39] (data not shown). Fig. 1 also shows that both nitrite reductase and glutamate synthase co-migrate with ferredoxin during gel filtration chromatography on LKB Ultrogel AcA34 in low ionic strength buffers. The apparent M_r values of the nitrite reductase and glutamate synthase complexes with ferredoxin were 110 kDa and 200 kDa, respectively. Chromatography of the enzymes under identical conditions as in Fig. 1, but in the absence of ferredoxin gave apparent molecular weights of 85 kDa and 180 kDa for nitrite reductase and glutamate synthase respectively. Addition of 200 mM NaCl to the elution buffers to raise the ionic strength eliminated the co-migration of ferredoxin with both nitrite reductase and glutamate synthase during gel filtration chromatography (data not shown). At high ionic strength a mixture of glutamate synthase and ferredoxin eluted from Ultrogel AcA34 as two separate components with M_r values of 140 000 (the apparent M_r of glutamate synthase, as determined by gel filtration chromatography, had previously been shown to vary with ionic strength [9]) and 11 000, respectively. At high ionic strength, a mixture of nitrite reductase and ferredoxin eluted as two separate components with M_r values of 85 000 and 11 000, respectively. These observations provide additional evidence for the importance of electrostatic forces in stabilizing the complex between ferredoxin and nitrite reductase [15,16] and suggest that the glutamate synthase-ferredoxin complex is also stabilized by electrostatic forces.

As formation of both the $NADP^+$ reductase · ferredoxin [10–14] and nitrite reductase · ferredoxin [15] complexes result in significant changes in

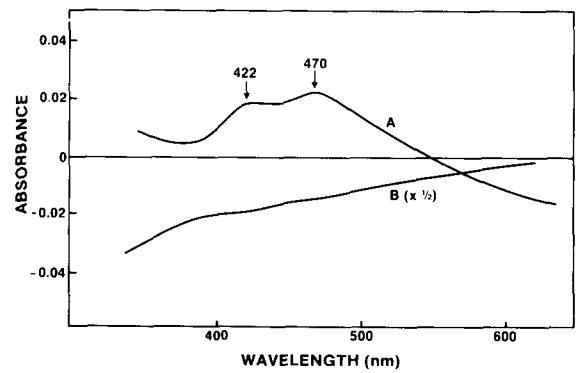

Fig. 2. The effect of glutamate synthase-ferredoxin interaction on the visible spectra of the proteins. (A) Native Ferredoxin. The sample cuvette contained in a 1.0 ml volume 25 μM glutamate synthase in 10 mM potassium phosphate buffer (pH 7.5), 12.5 mM 2-mercaptoethanol and 2 mM 2-oxoglutarate plus (in the same compartment) 50 μM native ferredoxin. The reference cuvette contained the same components but had ferredoxin and glutamate synthase in separate compartments. (B) Modified Ferredoxin. As in (A), but native ferredoxin was replaced by glycine ethyl ester plus EDC-modified ferredoxin.

the absorbance spectra of the proteins' prosthetic groups, it was of interest to determine whether complex formation between ferredoxin and glutamate synthase also produced spectral perturbations. Fig. 2A shows that absorbance changes do result from mixing ferredoxin and glutamate synthase in low-ionic strength buffer. The difference spectrum (spectrum of the complex minus the sum of the spectra of the two separate proteins) contains maxima at 422 and 470 nm. Mixing ferredoxin and glutamate synthase in the same buffer to which 200 mM NaCl had been added to raise the ionic strength produced no detectable absorbance changes (data not shown), an observation that provides additional evidence for the electrostatic nature of the glutamate synthase · ferredoxin complex. Data from titrations of the ΔA (460–540 nm) produced by the addition of increasing amounts of ferredoxin to a fixed amount of glutamate synthase fit a single hyperbolic binding isotherm with $K_d = 14.5$ μM or the glutamate synthase · ferredoxin complex (see Table I).

The role of ferredoxin carboxyl groups in complex formation with glutamate synthase and nitrite reductase was investigated using ferredoxin that had been reacted with glycine ethyl ester in the presence of EDC so as to modify 3–4 carboxyl side-chain groups [24]. In control experiments, as reported previously [24], this modification largely eliminated the ability of ferredoxin to produce complex-indicating spectral perturbations on mixing with $NADP^+$ reductase and also inhibited $NADP^+$ photoreduction by chloroplast membranes by approx. 80%. We have also demonstrated, in contrast to the pattern shown for native ferredoxin in Fig. 1, that ferredoxin modified with glycine ethyl ester plus EDC does not co-migrate with $NADP^+$ reductase during gel filtration chromatography on Sephadex G-75 (data not shown). Table I shows that this modification of ferredoxin carboxyl groups decreases the V_{max} to only 20% of that observed with native, unmodified ferredoxin for both the nitrite reductase and glutamate synthase catalyzed reactions. Table I also shows that while modification of ferredoxin did not affect the K_m for ferredoxin in the nitrite reductase catalyzed reaction, it did affect the apparent K_m for ferredoxin in the glutamate synthase catalyzed reaction. The apparent K_m is considerably less favorable in the case of modified ferredoxin: 84 μM compared to 2.0 μM for native ferredoxin. (Lineweaver–Burk plots of $1/V$ vs. $1/$[ferredoxin] for the glutamate synthase-catalyzed reaction deviated somewhat from linearity. Hill plots [40] gave Hill coefficients of 1.8 and 1.3 for native and modified ferredoxins, respectively.)

The kinetic data of Table I indicate that modified ferredoxin, while impaired in its ability to function as an electron donor to nitrite reductase and glutamate synthase, can still interact with both enzymes. Further evidence for such interaction comes from the observation that the modified ferredoxin still can co-migrate with either enzyme during gel filtration chromatography (data not shown). This interaction can also be detected through changes in the proteins' visible absorbance spectra. Fig. 2B shows the difference spectrum that arises from mixing glutamate synthase with modified ferredoxin. The difference spectrum, which contains no distinct peaks, differs considerably from that observed for native ferredoxin (Fig. 2A), indicating an altered protein: protein interaction. A plot of ΔA (400–480 nm) vs. the concentration of modified ferredoxin does fit a single hyperbolic binding isotherm, allowing a calculation of K_d for this complex. Table I shows that carboxyl modification of ferredoxin decreases its affinity for glutamate synthase almost 7-fold, with K_d increasing from 14.5 μM for

TABLE I

THE EFFECT OF FERREDOXIN MODIFICATION ON KINETIC AND BINDING PARAMETERS FOR NITRITE REDUCTASE AND GLUTAMATE SYNTHASE

V_{max} and K_m^{app} values were determined from enzyme assays as described in Experimental Procedures. 100% V_{max} values were 308 and 100 units for nitrite reductase and glutamate synthase, respectively. K_d values for glutamate synthase and nitrite reductase were determined in 10 mM potassium phosphate buffers at pH 7.5 and 7.7, respectively.

Enzymes	Electron donor	Relative V_{max} (%)	K^{app} (μM)	K_d (μM)
Glutamate synthase	native ferredoxin	100	2.0	14.5
	modified ferredoxin	20	84	100
Nitrite reductase	native ferredoxin	100	20	0.63
	modified ferredoxin	20	20	100

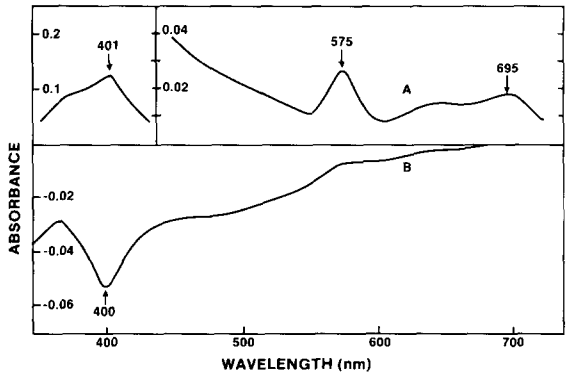

Fig. 3. The effect of nitrite reductase-ferredoxin interaction on the visible spectra of the proteins. (A) Native Ferredoxin. The sample cuvette contained in a 1.0 ml volume, 30 μM nitrite reductase in 10 mM phosphate buffer (pH 7.7) plus 30 μM native ferredoxin in the same compartment. The reference cuvette contained the same components but had ferredoxin and nitrite reductase in separate compartments. (B) Modified Ferredoxin. As in (A), but native ferredoxin was replaced by glycine ethyl ester plus EDC modified ferredoxin.

native ferredoxin to 100 μM for modified ferredoxin. Similar values for K_d were obtained using ΔA values from other wavelength pairs.

Despite the absence of any effect of ferredoxin modification on its K_m in the nitrite reductase catalyzed reduction of nitrite to ammonia, carboxyl modification appears to affect the binding of ferredoxin to nitrite reductase to an even greater extent than to glutamate synthase. Fig. 3B shows the difference spectrum resulting from the interaction between nitrite reductase and modified ferredoxin. For comparison, Fig. 3A shows the difference spectrum arising from the interaction of nitrite reductase with native ferredoxin. (The difference spectrum of Fig. 3A is essentially identical to that observed in an earlier study from our laboratory [15].) The difference spectra of Fig. 3A and B differ considerably, testifying to the altered interaction between the proteins that accompanies modification of the ferredoxin carboxyl groups. Titrating ΔA (575–605 nm) or ΔA (400–420) vs [ferredoxin] for the native and modified ferredoxins, respectively, produces two curves that both fit single hyperbolic binding curves. This data, summarized in Table I, shows that carboxyl group modification of ferredoxin increases the K_d for binding to nitrite reductase by a factor of approx. 160: from 0.63 μM for native ferredoxin (a value identical to that obtained in a previous study in our laboratory [14]) to 100 μM for the modified ferredoxin.

Discussion

The above results confirm our earlier data [15] for electrostatic complex formation between the native ($M_r = 85\,000$), ferredoxin-linked form of nitrite reductase and ferredoxin. This study also provides the first evidence for the formation of such a complex between ferredoxin and glutamate synthase. The addition of this third ferredoxin-dependent enzyme to the list of ferredoxin-linked enzymes known to form such complexes with ferredoxin (complexes for $NADP^+$ reductase and nitrite reductase having been documented earlier [10–16]) suggests the possible generality of the involvement of such complexes in the reactions of all ferredoxin-linked enzymes. It might be pointed out that such complexes may extend beyond the plant kingdom, as adrenodoxin, a Fe_2S_2-containing iron-sulfur protein similar in some ways to ferredoxin [41], forms an electrostatic complex with the flavoprotein adrenodoxin reductase [42–45] in the mammalian adrenal cortex.

Previous experiments had implicated carboxyl groups on both ferredoxin [22–24] and adrenodoxin [45] in supplying negative charges involved in electrostatic interactions with the flavoproteins $NADP^+$ reductase and adrenodoxin reductase, respectively. The data presented above suggest the possible generality of this phenomenon, in that carboxyl groups on ferredoxin also appear to be required for tight binding to two other chloroplast, ferredoxin-linked enzymes, nitrite reductase and glutamate synthase. The fact that modification of the same ferredoxin carboxyl groups inhibits binding to all three chloroplast enzymes raises the possibility that essentially the same portion of the ferredoxin molecule may be involved in binding $NADP^+$ reductase, nitrite reductase and glutamate synthase. (It had previously been proposed that ferredoxin might contain a common binding site for $NADP^+$ reductase and nitrite reductase [16].) However, quantitative differences between the effcts of modifying the 3–4 carboxyl residues on ferredoxin on binding the three en-

zymes (binding to $NADP^+$ reductase is virtually eliminated, while binding affinities to nitrite reductase and glutamate synthase are decreased by 160- and 7-fold, respectively) suggests some differences in binding sites. Ultimately it will be of greatest interest to identify the specific ferredoxin carboxyl residues involved in electrostatic complex formation with these three chloroplast enzymes. Preliminary peptide mapping experiments [46] suggest that the chemical modifications in ferredoxin that affect binding are localized in the regions of amino acid residues 26–30, 65–70 and 92–94. It may be that one or more of these regions of ferredoxin define, at least in part, the binding site for ferredoxin-linked enzymes.

Acknowledgements

This work was supported, in part, by a grant (to D.B.K.) from the U.S. Department of Agriculture (85-CRCR-1-1574).

References

1 Buchanan, B.B. and Arnon, D.I. (1979) Adv. Enzym. 33, 119–176
2 Shin, M. and Arnon, D.I. (1965) J. Biol. Chem. 240, 1405–1411
3 Hewitt, E.J. (1975) Annu. Rev. Plant Physiol. 26, 73–100
4 Hirasawa, M. and Tamura, G. (1980) Agric. Biol. Chem. 44, 749–758
5 Aketagawa, J. and Tamura ,G. (1980) Agric. Biol. Chem. 44, 2371–2378
6 Kreuger, R.J. and Siegel, L.M. (1982) Biochem. 21, 2892–2904
7 Wolosiuk, R.A. and Buchanan, B.B. (1977) Nature 266, 565–567
8 Lea, P.J. and Miflin, B.J. (1974) Nature 251, 614–616
9 Hirasawa, M. and Tamura, G. (1984) J. Biochem. 95, 983–994
10 Shin, M. and San Pietro, A. (1968) Biochem. Biophys. Res. Comm. 33, 38–42
11 Foust, G.P., Mayhew, S.G. and Massey, V. (1969) J. Biol. Chem. 244, 964–970
12 Nelson, N. and Neumann, J. (1969) J. Biol. Chem. 244, 1932–1936
13 Zanetti, G. and Curti, B. (1981) FEBS Lett. 129, 201–204
14 Batie, C.J. and Kamin, H. (1984) J. Biol. Chem. 259, 8832–8839
15 Hirasawa, M. and Knaff, D.B. (1985) Biochim. Biophys. Acta 830, 173–180
16 Privalle, L.S., Privalle, C.T., Leonardy, N.J. and Kamin, H. (1985) J. Biol. Chem. 260, 14344–14350
17 Shin, M. and Oshino, R. (1978) J. Biochem. 83, 357–361
18 Ida, S., Kobayakawa, K. and Morita, Y. (1976) FEBS Lett. 65, 305–308
19 Nakamura, S. and Kimura, T. (1971) J. Biol. Chem. 246, 6235–6241
20 Ricard, J., Nari, J. and Diamantidis, G. (1980) Eur. J. Biochem. 108, 55–66
21 Batie, C.J. and Kamin, H. (1981) J. Biol. Chem. 256, 7756–7763
22 Chan, T.-M., Ulrich, E.L. and Markley, J.L. (1983) Biochem. 22, 6002–6007
23 Zanetti, G., Aliverti, A. and Curti, B. (1984) J. Biol. Chem. 259, 6153–6157
24 Vieira, B. and Davis, D.J. (1985) Biochem. Biophys. Res. Comm. 129, 467–471
25 Hirasawa-Soga, M., Horie, S. and Tamura, G. (1982) Argric. Biol. Chem. 46, 1319–1328
26 Hirasawa, M., Fukushima, K., Tamura, G. and Knaff, D.B. (1984) Biochim. Biophys. Acta 791, 145–154
27 Tagawa, K. and Arnon, D.I. (1962) Nature 195, 537–543
28 Knaff, D.B., Smith, J.M. and Malkin, R. (1978) FEBS Lett. 90, 195–197
29 Davis, D.J. and San Pietro, A. (1977) Biochem. Biophys. Res. Comm. 74, 33–40
30 Davis, D.J. and Vieira, B.J. (1983) Biochem. Biophys. Res. Comm. 112, 508–514
31 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
32 Eguchi, Y. and Aso, Y. (1985) Biochemistry in BASIC, pp. 166–209, Kyoritsu Press, Tokyo
33 Matsubara, H., Hase, T., Wakabayashi, S. and Wada, K. (1980) in The Evolution of Protein Structure and Function (Sigman, D.S. and Brazier, M.A., eds.), pp. 245–266, Academic Press, New York
34 Shin, M. (1973) Biochim. Biophys. Acta 292, 13–19.
35 Andrews, P. (1965) Biochem. J. 96, 595–606
36 Keirns, J.J. and Wang, J.H. (1972) J. Biol. Chem. 247, 7374–7382
37 Gozzer, C., Zanetti, G., Galliano, M., Sacchi, G.A., Minichiotti, L. and Curti, B. (1977) Biochim. Biophys. Acta 485, 278–290
38 Sheriff, S., Teller, D.C. and Herriott, J.R. (1980) Arch. Biochem. Biophys. 205, 499–502
39 Karplus, P.A., Walsh, K.A. and Herriott, J.R. (1984) Biochem. 23, 6576–6583
40 Hammes, G.G. (1982) Enzyme Catalysis and Regulation, pp. 165–166, Academic Press, New York
41 Tanaka, M., Hainu, M., Kimura, T. and Yasunobu, K.T. (1973) J. Biol. Chem. 248, 1141–1157
42 Chu, J.-W. and Kimura, T. (1973) J. Biol. Chem. 248, 5183–5187.
43 Lambeth, J.D., Seybert, D.W. and Kamin, H. (1979) J. Biol. Chem. 254, 7255–7264
44 Hiwatashi, A., Ichikawa, Y., Maruya, T., Yamano, T. and Aki, K. (1976) Biochem. 15, 3082–3090
45 Geren, L.M., O'Brien, P., Stonehuerner, J. and Millett, F. (1984) J. Biol. Chem. 259, 2155–2160
46 Vieira, B.J., Colvert, K.K. and Davis, D.J. (1986) Biochim. Biophys. Acta 851, in the press