Disulfide Bond Formation in the Regulation of eIF-2α Kinase by Heme*

Jane-Jane Chen‡‡, Jane M. Yang‡, Ray Petryshyn¶, Nechama Kosower¶, and Irving M. London‡

From the Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, the Department of Biochemistry and Molecular Biology, State University of New York Health Science Center, Syracuse, New York 13210, and the Department of Human Genetics, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

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The inhibition of the autophosphorylation of the heme-regulated eukaryotic initiation factor (eIF)-2α kinase (HRI) by heme is very similar to that produced by thiold oxidation by diimide. The results obtained from the analysis of sodium dodecyl sulfate-polyacrylamide gel electrophoreses of phosphorylated and phosphorylated HRI under reducing and nonreducing conditions indicate that heme promotes disulfide formation in HRI. Hemin-promoted disulfide formation in HRI occurs under quasi-physiological conditions, i.e., 30 °C, 10 min at hemin concentrations of 5–10 μM. Under nondenaturing conditions, unphosphorylated HRI, phosphorylated HRI, hemin-treated unphosphorylated HRI, and hemin-treated phosphorylated HRI are all eluted identicaly on Sephacryl S-300 column chromatography with an apparent molecular mass of 290,000 daltons. It appears, therefore, that the disulfide formation caused by heme occurs within the unit of 290,000 daltons. In addition, heme treatment of phosphorylated HRI results in the appearance of a disulfide-linked form of higher molecular mass when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions. A similar high molecular mass form is observed when HRI is treated with 1,6-bismaleimidoheksane, a double sulfhydryl cross-linker agent, and the autophosphorylation of HRI and the phosphorylation of eIF-2α by HRI are greatly diminished; these effects are similar to the effects of heme on HRI. We conclude that disulfide formation by heme provides a likely mechanism by which heme prevents the activation and inhibits the activity of HRI.

Protein synthesis in intact reticulocytes and their lysates is dependent on the availability of heme. In heme deficiency, protein synthesis is inhibited at the level of initiation due to the activation of an inhibitor designated as heme-regulated inhibitor (HRI) (see the review (1)) or heme-controlled repressor (2, 3). HRI is a cyclic AMP-independent protein kinase which specifically phosphorylates the α subunit (eIF-2α) of the eukaryotic initiation factor 2 (eIF-2) (4–7). Phosphorylation of eIF-2α in reticulocyte lysates results in the binding and sequestration of reversing factor (RF, also designated as guanine nucleotide exchange factor) in a RF-eIF-2a complex; the unavailability of RF which is required for the exchange of GTP for GDP in the recycling of eIF-2 and in the formation of the ternary complex results in the cessation of the initiation of protein synthesis (8–16).

HRI is present in heme-supplemented reticulocyte lysates in an inactive form (pro-inhibitor); in heme deficiency (2, 7) or upon treatment with sulphydryl reagents such as N-ethylmaleimide or d-iodosobenzoate (17) HRI is activated. Activation of HRI is accompanied by its phosphorylation (for review see Ref. 1). It was previously suggested by Gross (18) that heme might prevent the formation of activated HRI by binding directly to the pro-inhibitor. The heme-reversible form of HRI has been purified (19), and the binding of heme directly to purified heme-reversible HRI has been demonstrated (20). However, the molecular mechanism by which heme regulates HRI was not further elucidated.

Since sulphydryl groups are involved in the activation of HRI, we have investigated the effect of the binding of heme on the thiol status of HRI. We report here that the binding of heme to HRI promotes disulfide bond formation in both phosphorylated and unphosphorylated HRI, that the inhibition of autophosphorylation of HRI by hemin is very similar to that produced by thiold oxidation by diimide, and that the cross-linking of sulphydryl groups by Bis-NEM produces effects similar to those of heme, i.e. diminished autokinase and eIF-2α kinase activity of HRI.

**EXPERIMENTAL PROCEDURES**

Materials—Acrylamide, N,N-methylenebisacrylamide, N,N′,N′,N′-tetramethylethylenediamine, β-mercaptoethanol, and molecular weight markers were obtained from Bio-Rad. N-Ethylmaleimide and diamide were purchased from Sigma. 1,6-Bis-maleimidohexane was provided by Pierce Chemical Co. Sephacryl S-300 and the molecular weight standards for column chromatography were obtained from Pharmacia LKB Biotechnology Inc. γ-[32P]ATP was provided by Du Pont-New England Nuclear.

Purification of Heme-reversible HRI—Postribosomal supernatant of reticulocyte lysates was the gift of Dr. William Merrick (Case Western Reserve University). The purification of HRI was performed as described previously (19) with the following modifications. Postribosomal supernatant was passed through a heparin-Sepharose column to remove RF and eIF-2. The flow-through material which contained HRI was concentrated by precipitation with ammonium sulfate to 50% saturation and was then further purified by DEAE-cellulose chromatography and phosphocellulose chromatography (19). Dithiothreitol (2 mM) was included in all buffers during the purification up to the step of phosphocellulose chromatography. Since the
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aim of this study was to investigate the thiol status of HRI, dithiothreitol was removed from the preparation of HRI during the Sephacryl S-300 column chromatography step. The heme-reversible autophosphorylation of HRI and the phosphorylation of eIF-2a were used as the criteria for localizing HRI throughout the purification procedure.

**Protein Kinase Assays; Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Autoradiography**—Protein kinase assays (20 μl) contained 20 mM Tris-HCl pH 7.7, 2 mM Mg(OAc)2, 40–60 mM KCl, 25–50 µM γ-[32P]ATP (20–30 Ci/mmole), and HRI preparation (50–100 ng). The phosphorylation reactions were initiated by the addition of γ-[32P]ATP; incubation was performed at 30 °C for 10 min. After incubation, the reactions were terminated by the addition of SDS-denaturing buffer (21) with or without β-mercaptoethanol as indicated. Samples denatured by SDS with β-mercaptoethanol were heated at 95 °C for 2–3 min while the samples denatured by SDS without β-mercaptoethanol were not heated. Proteins were separated by SDS-PAGE in 5, 7.5, and 10% polyacrylamide gel (acrylamide: bisacrylamide, 37.5:1), and the phosphoprotein profiles were analyzed by autoradiography.

**RESULTS**

**Effects of Thiol Reagents and Hemin on Autophosphorylation of HRI**—We have studied the effects of N-ethylmaleimide (NEM), GSSG, diamide, and hemin on the autophosphorylation and thiol status of partially purified HRI. The phosphorylation of HRI was terminated by the addition of SDS-denaturing buffer with or without disulfide reductant (i.e. β-mercaptoethanol), and the extent of the phosphorylation of HRI was analyzed by SDS-PAGE. The results are shown in Fig. 1. The addition of hemin or diamide results in decreased phosphorylation while the addition of NEM results in increased phosphorylation of HRI (Fig. 1B, tracks 2 and 5 versus track 1; track 3 versus track 1). The addition of GSSG appears to have no significant effect on the phosphorylation of HRI (Fig. 1B, track 4 versus track 1). All of the HRI preparations pretreated with these thiol reagents or hemin and then autophosphorylated migrate with an apparent molecular mass of 92 kDa in the presence of β-mercaptoethanol (Fig. 1B). In the absence of β-mercaptoethanol, the 32P radioactivity of the untreated HRI that migrates with an apparent molecular mass of 92 kDa is the same as in the presence of β-mercaptoethanol (Fig. 1A, track 1, and Fig. 1B, track 1). Similar observations are found with NEM-treated and GSSG-treated HRI (Fig. 1A, tracks 4 and 5 versus Fig. 1B, tracks 3 and 4). This result indicates that in the presence of SDS, HRI, whether untreated, NEM-treated, or GSSG-treated, is a monomer of 92 kDa. By contrast, in the absence of β-mercaptoethanol, hemin-treated and diamide-treated HRI have less radioactivity in the 92-kDa protein than they do in the presence of β-mercaptoethanol (Fig. 1A, tracks 2 and 6 versus Fig. 1B, tracks 2 and 5). This result indicates that both hemin and diamide promote the formation of disulfide bond(s) HRI. As a result of the formation of disulfide(s), HRI, in the absence of β-mercaptoethanol, migrates as a larger molecule, with an apparent molecular mass of 180 kDa (Fig. 1A, tracks 2 and 6). Diamide has been shown to oxidize thiols and to form disulfides in proteins (25). Our findings are consistent with this mechanism of action of diamide. The similar actions of hemin and diamide on the phosphorylation of HRI further support the view that heme, the physiological regulator of HRI, promotes disulfide bond formation in HRI.

**Hemin Promotes the Formation of Disulfides in Activated Phosphorylated HRI**—Since phosphorylated HRI has been shown to bind hemin (20), we have investigated the effects of hemin on the formation of disulfides in phosphorylated HRI. The results in Fig. 2 show that when hemin is added to phosphorylated HRI, there is a significant shift in mobility of 32P-labeled HRI from 88-kDa polypeptide to a region of 200-kDa polypeptides under nonreducing conditions (tracks 3 and 4). Note that the apparent molecular mass greater than 200 kDa is observed in 10% SDS-PAGE gel, whereas 5% SDS-PAGE gel yields a value of 180 kDa for the same polypeptide. Similarly an apparent molecular mass of 88 kDa is observed in the 10% gel, whereas it migrates as 92 kDa in a 5% gel. No difference in the migration of [32P]HRI is observed upon hemin treatment when analyzed by SDS-PAGE under reducing conditions (Fig. 2A, tracks 1 and 2). This observation indicates that hemin promotes the formation of disulfides in phosphorylated HRI and results in loss of 88-kDa HRI and the appearance of HRI with an apparent molecular mass of 200 kDa. The 88-kDa and higher molecular mass forms of HRI can be separated readily by SDS-PAGE under nonreducing conditions. However, under reducing conditions the disulfides are reduced to sulfhydryl, and the two forms of HRI behave identically. We have found that treatment of phosphorylated HRI with cobalt protoporphyrin IX also results in the formation of a disulfide-linked higher molecular mass form of HRI (Fig. 2B, track 3). Cobalt protoporphyrin IX is more effective than hemin in the promotion of intersubunit disulfide formation in HRI; there is a complete shift of HRI from 92 kDa to a higher molecular mass form. When a double sulfhydryl alkylating cross-linker, Bis-NEM (NEM-(CH2)4-NEM), is used, a similar high molecular mass form of phosphorylated HRI is formed (data not shown).

Disulfide bond formation promoted by hemin in HRI is dependent on the concentration of hemin (Fig. 3). The amount

**Fig. 1. Effect of thiol reagents on phosphorylation of HRI.** Protein kinase assays containing HRI were incubated with each thiol reagent for 5 min at 30 °C. The phosphorylation reactions were initiated by the addition of γ-[32P]ATP and were terminated by SDS-denaturing buffer as described under "Experimental Procedures." The autophosphorylation of HRI was analyzed by 7.5% SDS-PAGE and autoradiography. Panel A, nonreducing conditions (without β-mercaptoethanol); track 1, no addition; track 2, hemin (10 μM); track 3, dithiothreitol (DTT, 1 mM); track 4, NEM (1 mM); track 5, GSSG (1 mM); track 6, diamide (0.5 mM). Panel B, reducing conditions with β-mercaptoethanol; track 1, no addition; track 2, hemin (10 μM); track 3, NEM (1 mM); track 4, GSSG (1 mM); track 5, diamide (0.5 mM).
Hemin Promotes Disulfide Formation in Phosphorylated HRI

![Graph](image)

**Fig. 2.** Hemin promotes disulfide formation in phosphorylated HRI. HRI was phosphorylated as described in "Experimental Procedures." At the end of the protein kinase assay, NaF (50 mM) was added to prevent dephosphorylation of the HRI, and EDTA (2 mM) was added to stop further phosphorylation of HRI. Panel A, reducing conditions; Panel B, nonreducing conditions. Phosphorylated HRI was then treated with ethylene glycol (EG) (track 1), hemin (H) (10 μM, track 2), or cobalt protoporphyrin IX (CoPP) (10 μM, track 3) at 30 °C for 5 min. Disulfide formation was analyzed by 10% SDS-PAGE in the presence and absence of β-mercaptoethanol. Phosphorylated HRI was excised from the gel and quantitated by scintillation counting. For 88-kDa polypeptide in Panel A, EG 503 cpm, Hemin 438 cpm, CoPP 486 cpm. For 88 kDa in Panel B, EG 310 cpm, Hemin 175 cpm, CoPP 66 cpm. For 200 kDa in Panel B, EG 81 cpm, Hemin 199 cpm, CoPP 388 cpm.

of 32P-labeled HRI in the higher molecular mass region is quite broad, and it is difficult to quantitate the radioactivity in this region accurately. We have quantitated the loss of the 32P-labeled HRI in the 92-kDa region. In the presence of β-mercaptoethanol, the disulfides formed at various concentrations of hemin in the phosphorylated HRI are reduced, and therefore all the 32P-labeled HRI is recovered in the 92-kDa region as shown by the straight line (x--x) in Fig. 3. However, in the absence of β-mercaptoethanol there is a gradual loss of the 32P-labeled HRI in the 92-kDa polypeptide as the concentration of hemin is increased from 0 to 10 μM. Disulfide formation appears to be optimal at a hemin concentration of 5–10 μM. After incubation at 30 °C for 10 min, 50% of the phosphorylated HRI is converted to the disulfide form.

**Molecular Size of HRI**—Upon treatment with hemin or cobalt protoporphyrin IX, a disulfide-linked higher molecular mass form of HRI is formed when analyzed by SDS-PAGE under nonreducing conditions. To determine the molecular size of disulfide-linked and unmodified HRI under nondenaturing conditions, studies using column chromatography were performed. Under nondenaturing conditions, unphosphorylated HRI, phosphorylated HRI, hemin-treated HRI, and hemin-treated prephosphorylated HRI were all eluted identically from a Sephacryl S-300 column with an apparent molecular mass of 290 kDa. A typical pattern of elution of HRI from a Sephacryl S-300 column is shown in Fig. 4. The column fractions were analyzed by autoradiography of HRI and Western blot analysis; in all cases only one species of HRI was found. A similar observation was made after glycerol gradient fractionation of unphosphorylated HRI treated with hemin or diamide (data not shown). It appears that the formation of disulfide bonds achieved by hemin occurs within the unit of 290 kDa. Similar findings were made with Bis-NEM cross-linked HRI (Fig. 5).

**Treatment of Phosphorylated HRI with Hemin Inhibits the Cross-linking of HRI by Bis-NEM**—Since the covalently linked HRI is formed by treatment of HRI with Bis-NEM or hemin (Figs. 2, 4, and 5), we then asked whether pretreatment...
of HRI with hemin would influence the cross-linking of HRI by Bis-NEM. Since the Bis-NEM cross-linked HRI cannot be reduced by β-mercaptoethanol and remains as a higher molecular mass form (180 kDa) whereas the disulfide-linked HRI promoted by hemin can be reduced by β-mercaptoethanol to the 92-kDa monomer, it is possible to distinguish the two molecular mass forms by SDS-PAGE under reducing conditions.

The formation of the 180-kDa species of HRI after treatment with 20 or 100 μM Bis-NEM is shown in Fig. 6 (tracks 3 and 4). Since the solubility of Bis-NEM is very poor in aqueous solution, the Bis-NEM solution is prepared in dimethyl sulfoxide which does not promote the formation of the 180-kDa species (Fig. 8, track 2). When phosphorylated HRI is pretreated with hemin to promote disulfide formation, the formation of the 180-kDa dimer by Bis-NEM is inhibited and the 92-kDa monomer is retained (tracks 5 and 6). These results suggest that either hemin and Bis-NEM react with the same sulfhydryl groups of HRI or that the binding of hemin to HRI alters its conformation so that sulfhydryl groups which previously could react with Bis-NEM are now not accessible to Bis-NEM.

Effect of Bis-NEM on the Autophosphorylation of HRI and the Phosphorylation of eIF-2α—Since treatment with Bis-NEM, as with hemin, results in formation of 180-kDa HRI, does it affect the kinase activity of HRI? Results in Fig. 7A indicate that Bis-NEM at 20 or 100 μM abolishes HRI autokinase activity; dimethyl sulfoxide alone does not inhibit autophosphorylation. At 20 μM Bis-NEM completely cross-links the phosphorylated HRI (Fig. 6), with the result that there is very little autokinase activity in the Bis-NEM cross-linked 180 kDa species (Fig. 7A, track 3). Thus the inhibitory effect of Bis-NEM on the autokinase activity of HRI is very similar to that of hemin and diamide (Fig. 1).

We have observed that dimethyl sulfoxide (5%) inhibits completely the eIF-2α phosphorylation of HRI even though dimethyl sulfoxide does not affect HRI autophosphorylation. In order to test the eIF-2α kinase activity of Bis-NEM-treated HRI, a small aliquot of pretreated HRI was added to the eIF-2α kinase mix so that the final concentration of dimethyl sulfoxide was 0.25%. At this concentration of dimethyl sulfoxide eIF-2α kinase activity of HRI is not significantly affected by dimethyl sulfoxide (Fig. 7B, tracks 1, 6, and 7). The Bis-NEM cross-linked HRI, whether it is prephosphorylated or not, has very little eIF-2α kinase activity (Fig. 7B, tracks 2 and 3); this inhibition of eIF-2α kinase activity is very similar to that observed with hemin-treated HRI (tracks 4 and 5).

**DISCUSSION**

The results reported here demonstrate that hemin promotes disulfide formation in HRI under quasi-physiological conditions. We obtain an apparent molecular mass of 290 kDa for HRI on Sephacryl S-300 column chromatography. This is in agreement with the value obtained by Gross and Rabinovitz (17) and Hunt (23). In addition, there is no difference in the elution profile of heme-reversible HRI, whether or not it has been phosphorylated and whether or not it has been treated with hemin. This observation is consistent with earlier reports which indicate that there is no measurable change in the molecular size during the conversion of the proinhibitor to the activated inhibitor (17). HRI has been shown to have a
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**FIG. 7.** Effect of Bis-NEM on the phosphorylation of HRI and eIF-2α. A, HRI was treated with H2O (track 1), dimethyl sulfoxide (DMSO, 5% v/v, track 2), Bis-NEM (20 μM, track 3), and Bis-NEM (100 μM, track 4) and then phosphorylated as described in the legend of Fig. 1. DTT, dithiothreitol. B, HRI was pretreated with H2O (track 1), Bis-NEM (20 μM; track 2), hemin (10 μM, track 4), dimethyl sulfoxide (DMSO, 5%, track 6) and then phosphorylated as described in the legend of Fig. 1. HRI was prephosphorylated and treated with Bis-NEM (20 μM, track 3), hemin (10 μM, track 5), and dimethyl sulfoxide (5%, track 7) as described in the legend of Fig. 2. An aliquot of pretreated HRI (1 μl) was added to protein kinase reaction mixture which contains 0.5 μg of rabbit reticulocyte eIF-2, and the phosphorylation of eIF-2α was carried out as described under "Experimental Procedures." Phosphorylation of eIF-2α was analyzed by 10% SDS-PAGE.

**FIG. 8.** Schematic model of the covalent dimer formation of HRI by hemin and Bis-NEM.

A 180-kDa species of HRI has been reported upon cross-linking with dimethyl suberimidate (23). It is likely that HRI exists in the form of a dimer with a rather elongated shape. The disulfide bond formation promoted by hemin in HRI reported here occurs within the dimer, since no larger forms of HRI are detected. Furthermore, hemin treatment of phosphorylated HRI results in the appearance of a disulfide-linked 180-kDa species when analyzed by SDS-PAGE under nonreducing conditions. Bis-NEM, a double sulfhydryl cross-linker, also produces a 180-kDa HRI linked by six carbon chains. These in vitro findings may be summarized in the schema shown in Fig. 8. On treatment of HRI by hemin or Bis-NEM, a covalent dimer that has little or no autokinase or eIF-2α kinase activity is formed. In this schema, HRI is active when sulfhydryl groups are maintained in a reduced state or when disulfide formation is prevented.
by alkylation of the sulphydryl groups such as probably occurs at low concentrations of NEM (1mM) (17, 20). It seems likely that under physiologic conditions HRI is in an equilibrium between the active free sulphydryl state and the inactive disulfide state. Hemin and, to an even greater extent, cobalt protoporphyrin IX promote disulfide formation and inactivation of the enzyme. A deficiency of heme shifts the equilibrium to the free sulphydryl form of the enzyme that is active.

The experimental conditions of these in vitro studies are different from those of a protein-synthesizing reticulocyte lysate, and the applicability of this scheme to the regulation of HRI by heme under physiologic conditions in vivo remains to be determined. However, the disulfide-promoting effect of heme on unphosphorylated and phosphorylated HRI is consistent with these effects of heme in reticulocyte lysates: a) the inactivity of HRI in a hemin-supplemented lysate, b) the inactivation of HRI and the restoration of protein synthesis that occur on delayed addition of heme to a shut-off heme-deficient lysate, and c) the diminished inhibition of protein synthesis by hemin-treated phosphorylated HRI, compared with phosphorylated HRI, when added to a normal reticulocyte lysate (20).

We have identified a 92-kDa polypeptide (in a 5% SDS-PAGE gel) that binds ATP (unpublished observations) and has eIF-2α kinase and autokinase activities (Figs. 1 and 7). We have observed the inhibition of ATP binding to the 90-kDa polypeptide by heme (unpublished observation) and the inhibition of its autokinase and eIF-2α kinase activities by heme. Kudlicki et al. (24) have reported a 95-kDa polypeptide (in a 15% SDS-PGE gel) that binds ATP, has eIF-2α kinase activity, but is not phosphorylated (24). Their report does not indicate the effect of heme on either eIF-2α kinase activity or ATP binding. The apparent absence of autokinase activity in their preparation may be due to prior phosphorylation and activation of the polypeptide. The relationship of their observations to the findings in our study remains to be clarified.

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REFERENCES
1. London, I. M., Levin, D. H., Matts, R. L., Thomas, N. S. B., Petryshyn, R., and Chen, J.-J. (1987) in The Enzymes (Boyer, P. D. and Krebs, E. G., eds) Vol. XVIII, Part B, pp. 360-377, Academic Press, Orlando, FL
2. Maxwell, C. R., Kamper, C. S., and Rabinovitz, M. C. (1971) J. Mol. Biol. 68, 317-327
3. Gross, M., and Redman, R. (1987) Biochim. Biophys. Acta 908, 125-130
4. Farrell, P., Balkow, K., Hunt, T., Jackson, R. J., and Trachsel, H. Cell II, 187-200
5. Levin, D. H., Ranu, R. S., Ernst, V., and London, I. M. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3112-3116
6. Kramer, G., Cimadivella, M., and Hardesty, B. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3078-3082
7. Ranu, R. S., and London, I. M. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 4349-4353
8. Amesz, H., Goumans, H., Hanbrich-Morree, T., Voorma, H. O., and Benne, R. (1979) Eur. J. Biochem. 98, 513-520
9. Siekierka, J., Maurer, L., and Ochoa, S. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2537-2540
10. Matts, R. L., Levin, D. H., and London, I. M. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2559-2563
11. Fanniers, R., and Heashaw, E. C. (1983) J. Biol. Chem. 258, 7928-7934
12. Pain, V. M., and Clemens, M. J. (1983) Biochemistry 22, 726-733
13. Siekierka, J., Manne, V., and Ochoa, S. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 352-356
14. Matts, R. L., and London, I. M. (1984) J. Biol. Chem. 259, 6708-6711
15. Thomas, N. S. B., Matts, R. L., Petryshyn, R., and London, I. M. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 6998-7002
16. Matts, R. L., Levin, D. H., and London, I. M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1217-1221
17. Gross, M., and Rabinovitz, M. (1972) Biochim. Biophys. Acta 287, 340-362
18. Gross, M. (1974) Biochim. Biophys. Acta 340, 484-497
19. Trachsel, H., Ranu, R. S., and London, I. M. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3654-3658
20. Fagard, R., and London, I. M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 866-870
21. Laemmli, U. K. (1970) Nature 227, 680-685
22. Kosower, N. S., Kosower, E. M., Wertheim, B., Correa, W. S. (1969) Biochem. Biophys. Res. Commun. 37, 590-596
23. Hunt, T. (1979) Miami Winter Symp. 16, 321-346
24. Kudlicki, W., Fullilove, S., Read, R., Kramer, G., and Hardesty, B. (1987) J. Biol. Chem. 262, 9695-9701