Mechanism of Inhibition of Peptide Chain Initiation by Amino Acid Deprivation in Perfused Rat Liver

REGULATION INVOLVING INHIBITION OF EUKARYOTIC INITIATION FACTOR 2α PHOSPHATASE ACTIVITY*

(Received for publication, July 23, 1990)

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In previous studies, initiation of protein synthesis was shown to be inhibited in perfused rat livers deprived of single essential amino acids. In the present study, histidinol, a competitive inhibitor of histidinyl-tRNA synthetase, was used to amplify the effects of histidine deprivation on protein synthesis in perfused liver to facilitate investigation of mechanisms involved in the inhibition of peptide chain initiation. Protein synthesis was reduced to 77% of the control rate in livers deprived of histidine and to 13% of the control rate in livers deprived of histidine and exposed to 2.0 mM histidinol. Inhibition of protein synthesis caused by histidine deprivation alone was accompanied by a 2-fold increase in the number of free ribosomal particles, a 29% decrease in Met-tRNA, binding to 43 S preinitiation complexes, and a 31% reduction in activity of eukaryotic initiation factor 2B (eIF-2B). By comparison, histidine deprivation combined with histidinol addition resulted in a 3-fold increase in free ribosomal particles, a 66% decrease in Met-tRNA, binding, and a 78% reduction in eIF-2B activity. The proportion of the α-subunit of eukaryotic initiation factor two (eIF-2) in the phosphorylated form increased from 8.9 ± 0.8% in control livers to 52.4 ± 5.5% in response to histidinol. The increase in the amount of eIF-2α in the phosphorylated form apparently was not due to an increase in kinase activity, because there was no change in eIF-2α kinase activity in extracts of livers perfused with medium containing histidinol compared to controls. Instead, the increased phosphorylation of eIF-2α was associated with an inhibition of eIF-2α phosphatase activity. Thus, in contrast to other systems that have been examined, the mechanism involved in the increase in the phosphorylation state of eIF-2α appears to involve an inhibition of eIF-2α phosphatase activity rather than activation of an eIF-2α kinase.

The first step in peptide chain initiation is the binding of initiating methionyl-tRNA<sup>Met</sup> (Met-tRNA<sup>Met</sup>)<sup>1</sup> to the 40 S ribosomal subunit to form a 43 S preinitiation complex (reviewed in Refs. 1 and 2). The binding of Met-tRNA<sup>Met</sup> to the 40 S subunit is mediated by eukaryotic initiation factor 2 (eIF-2) through formation of a ternary complex consisting of eIF-2, Met-tRNA<sup>Met</sup>, and GTP. Following addition of mRNA and the 60 S ribosomal subunit to the 43 S preinitiation complex, GTP bound to eIF-2 is hydrolyzed and eIF-2 is released as a binary complex containing GDP. Before eIF-2 can bind Met-tRNA<sup>Met</sup> and participate in another round of initiation, the GDP bound to eIF-2 must be exchanged for GTP. Another initiation factor, eukaryotic initiation factor 2B (eIF-2B),<sup>2</sup> catalyzes the exchange of GDP bound to eIF-2 for GTP, allowing eIF-2 to re-form the ternary complex. In rabbit reticulocyte lysate deprived of hemin, an inhibition of protein synthesis develops that is characterized by a disaggregation of polysomes and an increase in the number of free ribosomal particles. Prior to the onset of inhibition, there is activation of a cyclic AMP-independent protein kinase (the heme-controlled repressor or HCR) that phosphorylates the α-subunit of eIF-2. The affinity of eIF-2B for eIF-2 phosphorylated on the α-subunit (eIF-2αP) is much higher than that for nonphosphorylated eIF-2 resulting in the sequestration of eIF-2B into an inactive eIF-2B-eIF-2αP complex.

An inhibition of peptide chain initiation is associated with increased phosphorylation of eIF-2α in a number of different cell types in response to such diverse stimuli as heat shock (3, 4), heavy metals (5), viral infection (6), and deprivation of heme (7), amino acids (4), glucose (4), insulin (8), or serum (4). However, the mechanism responsible for the increased phosphorylation of eIF-2α is known in only three cases: 1) heme deprivation of rabbit reticulocyte lysate (reviewed in Refs. 2 and 7), viral infection (reviewed in Ref. 6), and 3) heavy metal treatment of reticulocyte lysate (5). In all three cases, the increased phosphorylation of eIF-2α is associated with activation of an eIF-2α kinase. In two cases, heme deprivation and heavy metal treatment of reticulocyte lysate,

<sup>1</sup>The abbreviations used are: Met-tRNA<sup>Met</sup>, initiation form of methionyl-tRNA<sup>Met</sup>; eIF-2B, eukaryotic Initiation factor 2B; eIF-2, eukaryotic Initiation factor 2; HEPES, N-2-hydroxyethylpiperazine-N' -2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; MOPS, morpholinopropanesulfonic acid; ApA, adenosine 5',5'-diphosphate; P<sub>4</sub>PP<sub>4</sub>, tetraphosphate; SDS, sodium dodecyl sulfate; HCR, heme-controlled repressor; HPLC, high performance liquid chromatography.

<sup>2</sup>The nomenclature used in this report for protein synthesis initiation factors follows the guidelines suggested by the Nomenclature Committee of the International Union of Biochemistry (1989) Eur. J. Biochem. 186, 1-3. The factor termed eIF-2B in the current study was referred to as the guanine nucleotide exchange factor, or GEF, in our earlier work (e.g. Ref. 18) and in the work of others (e.g. Refs. 21 and 27).
the kinase activated is HCR. Viral infection has been shown to activate a second eIF-2α kinase, the double-stranded RNA-activated inhibitor.

In the present study, we have investigated the mechanism of inhibition of peptide chain initiation induced by amino acid deprivation in perfused rat livers using a competitive inhibitor of the aminocyclation of tRNA^His, histidinol (9), to amplify the effects of histidine deprivation. We show that protein synthesis is inhibited at the level of peptide chain initiation with disaggregation of polysomes and decreased formation of 43 S preinitiation complexes. In addition, we show that the inhibition of peptide chain initiation involves phosphorylation of the α-subunit of eIF-2 with a concomitant reduction in eIF-2B activity. Most importantly, we show that, unlike heme deprivation of rabbit reticulocyte lysate or viral infection, the increased proportion of eIF-2α present in the phosphorylated state is not due to stimulation of an eIF-2α kinase. Instead, the increased phosphorylation of eIF-2α is associated with a reduction in eIF-2α phosphatase activity.

**EXPERIMENTAL PROCEDURES**

*Animals*—Male Sprague-Dawley rats were maintained on a 12-h light/12-h dark cycle with food and water provided ad libitum. Rats weighing 100–150 g were anesthetized prior to experiments with an intraperitoneal injection of sodium pentobarbital (8 mg/100 g).

*Liver Perfusion*—Livers were perfused in situ as described previously (10) with the following modifications. Livers were perfused for 30 min with a nonrecirculating medium delivered at a flow rate of 7 ml/min. The perfuse contained amino acids present at either 10 times their concentrations in rat arterial plasma (11) (except leucine, which was present at 5 mM throughout) (referred to as control medium), 10 times the concentrations found in rat arterial plasma except for histidine (referred to as histidine-deprived medium), or 10 times the concentration in rat arterial plasma except for histidine and containing additionally 2 mM histidinol (referred to as histidinol medium). For determination of rates of protein synthesis, the livers were perfused for an additional 15 min with medium containing [3H]leucine (1 μCi/ml).

**Measurement of Rates of Total Protein Synthesis**—Rates of synthesis of total liver proteins were measured as described previously (12) except that livers were pulse-labeled with [3H]leucine for 15 min rather than 10 min. The 15-min labeling time used in the present study provided an estimate of total protein synthesis because newly synthesized secretory proteins still would be retained in the liver (13).

*Isolation of Ribosomal Subunits on Sucrose Density Gradients*—Ribosomal subunits were isolated by sucrose density gradient centrifugation as described by Delaunay et al. (17). For isolation of 40 S subunits, ribosomes were homogenized in a Dounce homogenizer on ice in 7 volumes of Buffer A containing 24 mM HEPES, pH 7.5, 250 mM KCl, 1 mM magnesium acetate, 5 mM dithiothreitol, and 250 mM sucrose with 0.1 mM EDTA. Following centrifugation, supernatant samples (1.0 ml) were layered onto 25–68% exponential sucrose density gradients made up in Buffer A and centrifuged at 35,000 rpm in an SW 41 rotor for 21 h at 4 °C. The A260 of the gradients was monitored, and 0.3-ml fractions were collected for determination of density as described by Henshaw (14). Each fraction was examined for [35S]methionyl-tRNA as described previously (15).

**Measurement of eIF-2B Activity in Extracts Prepared from Perfused Rat Livers—**eIF-2B activity was measured exactly as described by Kimball et al. (16).

*Determinition of the Phosphorylation State of eIF-2α—*The state of phosphorylation of the α-subunit of eIF-2 was determined by protein immunoblot analysis of isoelectric focusing gels as described previously (16). The criteria used to assign a phosphorylation state of eIF-2α separated by isoelectric focusing as differing by phosphorylation were 3-fold. First, the modified protein migrated to the same position on isoelectric focusing slab gels as did liver eIF-2α phosphorylated using the reticulocyte kinase HCR. Second, the modified protein contained an amino acid signature with histidinol treatment using HCR and [32P]ATP. Finally, we have shown previously that [32P] incorporation into eIF-2α was increased in hepatocytes deprived of essential amino acids (55). The modified form of eIF-2α present in livers perfused with histidinol medium migrated identically on two-dimensional gels with the modified protein from livers perfused with histidine-deprived medium.

**Determination of eIF-2α Kinase Activity in Liver Extracts—**The assay used to determine eIF-2α kinase activity in liver extracts was a modification of the assay described by Delaunay et al. (20). Livers were perfused with either control or histidinol medium as described above. Following perfusion, livers were homogenized in 7 volumes of Buffer A containing 50 mM Tris, pH 7.6, 75 mM KCl, 5 mM magnesium acetate, 6 mM β-mercaptoethanol, and 250 mM sucrose and then were centrifuged at 10,000 × g for 10 min at 2 °C. Aliquots of the supernatants (10 μl) were added to plastic tubes that contained 40 μl of a solution consisting of 4–5 μg of purified liver eIF-2 (21), 1.25 mM [γ-32P]ATP (2000 Ci/mol), 250 mM sucrose, 0.1 mM EDTA, and 0.375 M Tris, pH 8.8, and incubated for 5 min. An aliquot of each sample was then subjected to SDS-polyacrylamide gel electrophoresis as described previously (21). The stained gel was dried and exposed to Kodak XAR film at −70 °C for 3 h. The film was developed according to the manufacturer's instructions and then scanned using a Beckman DU-8B spectrophotometer.

**Determination of eIF-2α[P] Phosphatase Activity in Liver Extracts—**eIF-2 was purified from rabbit reticulocyte lysate by the method used for purification of the protein from rat liver (21). Following purification, eIF-2 (130 μg) was phosphorylated using the eIF-2α kinase assay described above. The modified form of eIF-2α present in the reaction mixture was removed and placed in a Eppendorf microfuge tube containing 10 μl of stop buffer (at 0 °C) (2.9% [w/v] glycerol, 6 mM Mg(OAc)₂, 0.575 M Tris, pH 8.8) and incubated for 5 min. An aliquot of each sample was then subjected to SDS-polyacrylamide gel electrophoresis as described previously (21). The stained gel was dried and exposed to Kodak XAR film at −70 °C for 3 h. The film was developed according to the manufacturer's instructions and then scanned using a Beckman DU-8B spectrophotometer.
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Perfused with control medium or histidinol medium. The livers were homogenized in 1 volume of ice-cold Buffer F (45 mM HEPES, pH 7.4, 90 mM potassium acetate, 75 mM EDTA, 375 mM magnesium acetate, 10% glycerol, and 2.5 mg/ml digitonin) and centrifuged at 10,000×g for 10 min at 2°C. The supernatants (35 μl) were added to plastic tubes which contained 215 μl of a solution consisting of 5 μg of eIF-2α(32P), 149 mM MOPS, pH 7.4, 52 mM KCl, 0.52 mM dithiothreitol, 1.05 mM magnesium acetate, and 0.1 mg/ml bovine serum albumin. The tubes were placed immediately in a water bath at 70°C. At various times, 50 μl of the reaction mixture were removed and placed in an Eppendorf microcentrifuge tube containing 30 μl of stop buffer (at 70°C). Samples were subjected to SDS-polyacrylamide gel electrophoresis and subsequent analysis as described above for determination of eIF-2α kinase activity.

**Table I**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Additions</th>
<th>Protein synthesis</th>
<th>% Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>15.0 ± 0.6 (9)</td>
<td>77</td>
</tr>
<tr>
<td>Histidine-deprived</td>
<td>None</td>
<td>11.6 ± 0.5* (8)</td>
<td>37</td>
</tr>
<tr>
<td>Histidine-deprived</td>
<td>Histidinol</td>
<td>1.9 ± 0.2* (9)</td>
<td>13</td>
</tr>
</tbody>
</table>

a *p < 0.0005 versus supplemented condition.

FIG. 1. Effects of histidine deprivation and histidinol treatment on ApA(40S) profiles of ribosomal subunits isolated on sucrose density gradients. Livers were perfused with either control medium (solid line), histidine-deprived medium (dashed line), or histidinol medium (dotted line). Following perfusion, livers were homogenized in 7 volumes of buffer and subunit profiles were determined by sucrose density gradient centrifugation as described under "Experimental Procedures." The positions of the 40 and 60 S ribosomal subunits are noted in the figure.

Results

Rates of protein synthesis were measured in livers perfused with control medium, histidine-deprived medium, and histidine-deprived medium containing histidinol (Table I). When perfused livers were deprived of a single, essential amino acid (histidine), protein synthesis was reduced to 77% of the control rate. Addition of histidinol to the histidine-deprived medium caused an additional inhibition such that protein synthesis was reduced to only 13% of the rate observed in livers perfused with control medium.

In previous studies, the decreased rate of protein synthesis observed in livers perfused with amino acid-deficient medium was accompanied by a disaggregation of polysomes and an increase in concentration of ribosomal subunits and monomers (12). The inhibition of protein synthesis was determined by the incorporation of [3H]leucine into total protein during the final 15 min of perfusion and was expressed as milligrams of protein synthesized/g of cell protein/h (12). The values represent the average ± standard error for (N) experiments.

Livers were perfused in situ for 45 min as described under "Experimental Procedures." Histidinol, when present, was at a final concentration of 2 mM from the start of perfusion. Rates of protein synthesis were determined by the incorporation of [3H]leucine into total protein during the final 15 min of perfusion and was expressed as milligrams of protein synthesized/g of cell protein/h (12). The values represent the average ± standard error for (N) experiments.

Histidine-deprived medium caused an additional 2-fold increase in the rate of elongation (24). To examine whether histidinol treatment of perfused livers also resulted in an inhibition of peptide chain initiation, the accumulation of ribosomal subunits was examined by sucrose density gradient centrifugation. After 45 min of perfusion, livers were homogenized under conditions that reduce trapping of ribosomal particles in the postmitochondrial pellet and maximize recovery of free 40 and 60 S ribosomal subunits (25, 26). The postmitochondrial supernatant was then centrifuged through exponential sucrose density gradients. The absorbance profiles from the fractionated gradients are shown in Fig. 1. The content of subunits in livers perfused with histidine-deprived medium was approximately 2-fold greater than the content of livers perfused with control medium. Addition of histidinol to the histidine-deprived medium resulted in an even larger increase in subunit content to 3-fold more than that observed in livers perfused with control medium. Rates of protein synthesis in the livers used for subunit analysis yielded values similar to those shown in Table I.

The effect of histidinol on formation of 43 S preinitiation complexes in perfused livers was examined by measuring [35S]methionine binding to 40 S ribosomal subunits. Livers, perfused with either control, histidine-deprived, or histidinol
medium, were homogenized under conditions that maximize binding of methionyl-tRNA\[^{35}\text{S}\] to the 40 S subunit (14, 27).

The 40 S subunit fraction was isolated by sucrose density gradient centrifugation and further resolved by CsCl density gradient centrifugation (Fig. 2). In contrast to the results shown in Fig. 1, when ribosomal subunits were isolated in 25 mM KCl rather than 250 mM KCl, the content of 40 and 60 S ribosomal subunits did not change when livers were perfused with different media. However, the content of 40 S monomers increased 1.6- and 3.2-fold in livers perfused with histidine-deprived medium and histidinol medium, respectively, compared to livers perfused with control medium. Further resolution of the isolated 40 S subunits on CsCl density gradients revealed a significant difference among the three types of liver samples in the proportion of 40 S subunits having a density of 1.41 g/cm\(^3\) compared to those having a density of 1.48 g/cm\(^3\). The ratio of 1.41 to 1.48 species was reduced to 75% of the control condition in livers perfused with histidine-deprived medium and to 38% of the control condition in livers perfused with histidinol medium.

Labeling with [\(^{35}\text{S}\) ]methionine during the perfusion resulted in radioactivity being localized exclusively on the 1.41 g/cm\(^3\) species of the 40 S subunit (Fig. 2). The decrease in binding of [\(^{35}\text{S}\) ]methionine to the 1.41 g/cm\(^3\) species in livers perfused with medium deprived of histidine or additionally containing histidinol was proportional to the decrease in the ratio of 1.41 to 1.48 g/cm\(^3\) species. The amount of radioactivity associated with the 1.41 g/cm\(^3\) form was reduced to 71% of the control value in livers perfused with histidine-deprived medium and to 34% of the control value in livers perfused with histidinol medium.

In livers perfused with medium deprived of all amino acids except leucine, there was an inhibition of protein synthesis that was characterized by a disaggregation of polysomes and with decreased formation of 43 S preinitiation complexes (12). In livers perfused with medium deprived of histidine or additionally containing histidinol was proportional to the decrease in the ratio of 1.41 to 1.48 g/cm\(^3\) species. The amount of radioactivity associated with the 1.41 g/cm\(^3\) form was reduced to 71% of the control value in livers perfused with histidine-deprived medium and to 34% of the control value in livers perfused with histidinol medium.

The increase in state of phosphorylation of the α-subunit of eIF-2 in response to histidinol could be due to either an increase in the activity of an eIF-2α kinase, a decrease in activity of an eIF-2α phosphatase, or both. In order to determine whether or not the amount of eIF-2α present in the phosphorylated form changed in response to histidinol, phosphorylated and unphosphorylated eIF-2α present in livers perfused with either control or histidinol medium were separated by isoelectric focusing, and the relative amounts of each form were determined by protein immunoblot analysis (Fig. 4). The proportion of eIF-2α present in the phosphorylated form was increased significantly (\(p < 0.0025\)) in livers perfused with histidinol medium (52.4 ± 5.5%; \(n = 3\)) compared with livers perfused with control medium (8.9 ± 0.8%; \(n = 3\)).

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pared to control livers (17.0 ± 2.7 min⁻¹; n = 3).

Since there was no change in eIF-2α kinase activity in extracts from livers perfused with histidinol medium, the increase in the proportion of the α-subunit of eIF-2 present in the phosphorylated form most likely was due to a change in eIF-2α phosphatase activity. Therefore, eIF-2α phosphatase activity was measured in postmitochondrial supernatants from livers perfused with either control medium or histidinol medium. As shown in Fig. 6, eIF-2α(P) was readily dephosphorylated by either of the liver extracts. However, the time required to dephosphorylate one-half of the eIF-2α(P) (t½) using the extract from livers treated with histidinol (13.1 ± 0.8 min) was significantly greater (p < 0.001) than the t½ determined for the extracts from livers perfused with control medium (7.1 ± 0.8 min). The results suggest that the increased phosphorylation of eIF-2α in livers treated with histidinol was due to an inhibition of eIF-2α phosphatase activity rather than to an increase in an eIF-2α kinase activity.

The dinucleotide ApA is synthesized by aminoacyl-tRNA synthetases in mammalian cells (28–33). Several studies have shown a correlation between ApA content and cell proliferation (34–36). In addition, ApA is an effective inhibitor of the protein kinase activity of Rous sarcoma virus transforming protein pp60src (37). In order to determine whether ApA might be acting as a signal in the histidinol-induced inhibition of protein synthesis in liver, ApA levels were measured in extracts from livers perfused with control medium for 10 min followed by perfusion with medium containing histidinol for various times. As shown in Table II, the content of ApA gradually decreased as the length of exposure to histidinol medium increased. However, in contrast to the effect on protein synthesis, which was rapid and large, the decline in ApA content in response to histidinol was protracted and of lesser magnitude. Therefore, it seems unlikely that ApA was acting as a signal in generating the protein synthetic response to histidinol in liver.

DISCUSSION

In the present study, we have shown that livers perfused with either histidine-deprived medium or histidine-deprived medium containing histidinol exhibit an inhibition of protein synthesis that is characterized by a reduction in the rate of peptide chain initiation relative to elongation. This finding is not unique to liver. Other cell types, such as amino acid-deprived Ehrlich ascites cells (38) and histidinol-treated HeLa cells (39) also demonstrate an inhibition of peptide chain initiation relative to elongation. The basis for the inhibition of peptide chain initiation caused by amino acid deprivation has not been elucidated. It has been suggested that an accumulation of uncharged tRNA during amino acid deprivation leads to the inhibition of protein synthesis. Indeed, both deprivation of essential amino acids and inhibition of histidinyl-tRNA synthetase by histidinol have been shown to result in decreases in the proportion of tRNA which is aminoacylated (reviewed in Ref. 40). However, studies performed using extracts of Ehrlich ascites cells show no obvious relationship between the extent of tRNA charging and initiation of protein synthesis (41). Other studies also suggest that the rate of peptide chain initiation is not related to the extent of tRNA charging, but it may be coupled instead to the activity of the aminoacyl-tRNA synthetases (42, 43). Furthermore, it
inhibition of tRNA charging do not inhibit initiation, but inhibit elongation instead (44, 45). Also, histidinol inhibits the elongation rate by 50% in Krebs II ascites cells (46).

Whereas little is known about the signal that causes the inhibition of peptide chain initiation in amino acid-deprived cells, recent work using temperature-sensitive Chinese hamster ovary cell mutants (tsH1) has revealed significant details about the mechanism controlling the response to amino acid deprivation (41–43). These cells have a mutation in the leucyl-tRNA synthetase, and incubation of the cells at the nonpermissive temperature results in an inhibition in the charging of leucyl-tRNA by the synthetase. The mechanism proposed for the inhibition of protein synthesis observed in tsH1 when the incubation temperature is raised to the nonpermissive temperature (42) is based on the mechanism described for hemin-deprived rabbit reticulocyte lysate (reviewed in Refs. 1 and 2). In reticulocyte lysate deprived of hemin, a kinase is activated that phosphorylates the α-subunit of eIF-2. Phosphorylated eIF-2 forms a stable complex with eIF-2B, effectively sequestering eIF-2B into an inactive complex. Even though only about 30% of the eIF-2 present becomes phosphorylated in hemin-deprived reticulocyte lysate (47), essentially all of the eIF-2B is removed into an eIF-2a(P)-eIF-2B complex because the amount of eIF-2B is only about 20% of that of eIF-2. In the current study, we show that in livers perfused with medium containing histidinol, eIF-2B activity is significantly lower than in livers perfused with either control or histidine-deprived media. Also, the inhibition of eIF-2B activity, based on the amount of GDP exchanged in the first minute of incubation, is proportional to the inhibition of protein synthesis and the inhibition of Met-tRNA\(^{Met}\) binding to 40 S subunits. These observations suggest that the activity of eIF-2B is related directly to the observed rate of protein synthesis.

In an attempt to delineate the mechanism involved in the increased phosphorylation of eIF-2α in tsH1 cells placed at the nonpermissive temperature, both eIF-2α kinase and phosphatase activities were measured in cell extracts (43). In agreement with the results observed in the current study, eIF-2α kinase activity was not increased in extracts from cells placed at the nonpermissive compared to the permissive temperature. However, in contrast to the results presented in the current study, there was also no significant change in the activity of eIF-2α phosphatase in extracts from tsH1 cells incubated at the nonpermissive temperature. The basis for the difference between the eIF-2α phosphatase results presented here compared to the findings in tsH1 cells is unknown. It may be due to a difference in the systems themselves. For example, it was proposed that in tsH1 cells the phosphorylation state of eIF-2α increased in the intact cell as the result of a change in the accessibility of the substrate to an eIF-2α kinase and/or phosphatase rather than an actual change in enzyme activity. It should also be noted that the eIF-2α phosphatase assay used in the present study is significantly different than the assay used by Pollard and co-workers (43). Thus, the difference in results between that study and this one may be attributable to a difference in the method used to measure eIF-2α phosphatase activity.

Reticulocyte lysate has a potent eIF-2α phosphatase activity. The eIF-2α phosphatase was first isolated from rabbit reticulocytes by Crouch and Safer (48) and was found to be composed of two subunits of \(M, 60,000\) and 38,000. Further characterization showed that this phosphatase could be classified as a type \(2A\) enzyme (49). Protein phosphatase-\(2A\) is also present in rat liver (50). Although eIF-2α(P) has not been tested as a substrate for the purified rat liver enzyme,
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protein phosphatase-2A purified from rabbit liver has been shown to be as effective at dephosphorylating eIF-2α(P) as is the reticulocyte enzyme (49).

The mechanism involved in the regulation of protein phosphatase-2A activity in vivo is unknown. However, in vitro, the activity of protein phosphatase-2A is altered by the interaction of metabolites with substrates (51). For example, the binding of glucose to hepatic phosphorylase a stimulated its activity of protein phosphatase-2A.

In contrast, formation of either an eIF-2-GTP binary complex (52) or eIF-2-GTP-Met-tRNA ternary complex (53) had no effect on the rate of dephosphorylation of eIF-2α(P) by rabbit reticulocyte protein phosphatase-2A. In fact, the mechanism for the increased phosphorylation of eIF-2α regulated by a change in phosphatase activity rather than an increase in eIF-2α kinase activity. This is the first reported instance in which eIF-2α phosphorylation is altered at the level of peptide chain initiation in rat livers perfused with medium either lacking the essential amino acid histidine or lacking histidine and containing histidinol.

The characteristics of the inhibition are identical under the two conditions; however, the inhibition of protein synthesis and the corresponding changes in the translational apparatus are amplified in histidinol-treated liver compared to histidine deprivation alone. We have also shown that the probable basis for the inhibition of protein synthesis is the result of an increase in the phosphorylation of eIF-2α with a concomitant decrease in eIF-2B activity. Most importantly, we have shown that the mechanism for the increased phosphorylation of eIF-2α involves an inhibition of eIF-2α phosphatase activity rather than an increase in eIF-2α kinase activity. This is the first reported instance in which eIF-2α phosphorylation is regulated by a change in phosphate activity rather than an alteration in eIF-2α kinase activity.

Acknowledgments—We thank Drs. Richard Panniers and Edgar C. Henshaw of the University of Rochester Cancer Center, Rochester, NY, for generously providing the monoclonal antibody to eIF-2α that was used in these studies. In addition, we are grateful to Lynne Pletcher, Sharon Rannels, and Jill Wolfgang for fine technical assistance and to Lynne Pletcher and Sharon Rannels for help in preparation of the figures.

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