Characterization of the Hypoxia-inducible Protein Binding Site within the Pyrimidine-rich Tract in the 3′-Untranslated Region of the Tyrosine Hydroxylase mRNA

(Received for publication, September 12, 1995, and in revised form, November 2, 1995)

Maria F. Czyzyk-Krzeska‡ and John E. Beresh

From the Department of Molecular and Cellular Physiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267-0576

Reduced tension of O₂ slows the degradation rate of mRNA for tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis, in the pheochromocytoma (PC12) clonal cell line. The observed increase in half-life (30 h versus 10 h) correlates with enhanced binding of a 66-kDa protein (hypoxia inducible protein) to the pyrimidine-rich tract located between bases 1552-1578 in the 3′-untranslated region of TH mRNA (hypoxia-inducible protein binding site (HIPBS)). The present study investigates the protein binding site within the 27-base HIPBS, first by using specific cleavages of HIPBS and its flanking sequences with antisense oligodeoxynucleotides and RNase H and then by using mutational analysis of the binding properties. We found that the 27-base HIPBS oligoribonucleotide was sufficient to bind the protein in vitro in a hypoxia-stimulated manner. We further identified the optimal hypoxia-inducible protein binding site that is represented by the motif (U/C)(C/U)CCCU, where the core binding site is indicated by the underlined cytidines. Substitutions of either one of the cytidines with purine or uridine abolished the protein binding. The mutations within HIPBS, which partially reduced binding, did not prevent stimulation of protein binding for extracts from hypoxic cells. The hypoxia-induced increase in complex formation was proportional to the strength of binding using proteins from normoxic cells. The HIPBS element is conserved in TH mRNAs derived from different species.

It is well established that pyrimidine-rich sequences and specific polypyrimidine (poly(Y))-binding Proteins represent important functional elements in RNA metabolism (1). Among the best characterized pyrimidine-rich motifs are the poly(Y) tracts located between the branch site and the 3′ splice site of introns. These tracts are known to regulate the processing of intervening sequences (2). Other examples of pyrimidine-rich motifs include oligopyrimidine sequences in the 5′-untranslated regions of mRNAs for ribosomal proteins that regulate translation of these proteins (3–5) and AUUUA pentamers, which are well established as destabilizing elements in the 3′-untranslated regions of different short-lived mRNAs (6). Recently, the poly(Y) tracts in the 3′-untranslated region of 15-lipoxygenase mRNA were shown to regulate its translation (7). A number of pyrimidine-rich tract-binding proteins have been recently identified, purified, and cloned. Examples of these include the poly(Y) tract-binding protein (8), the essential splicing factor, U2AF (9); the splicing regulator Sex-lethal, Sxl (10); the group of heterogeneous nuclear ribonucleoprotein particles (hnRNPs) such as the proteins C1 and C2 (11), I (12), and K and J (13); and the 48-kDa protein binding to 15-lipoxygenase 3′-UTR (7). Importantly, various pyrimidine-rich tract-binding proteins show affinities for distinct binding motifs within the pyrimidine-rich sequences. In this respect the proteins K and J bind to polycytidines sequences (13), and proteins C1 and C2 bind to the polypuridines (11) sequences. The distinct consensus binding sequences were also found to be preferred by the poly(Y) tract-binding, Sxl, and U2AF proteins (14).

We have recently reported that stability of mRNA for tyrosine hydroxylase (EC 1.14.16.2), the regulatory enzyme in the synthesis of catecholamines, a major group of neurotransmitters, is three times increased during hypoxia in the pheochromocytoma-derived PC12 clonal cell line (15). This is associated with increased TH protein synthesis (16) and release of the neurotransmitter dopamine during hypoxia (17). The effect of hypoxia on TH mRNA stability is specific because neither β-actin nor GAPDH mRNAs are stabilized, and it represents a novel and important mechanism by which environmental factors can affect gene expression. We have since found that this increase in TH mRNA half-life during hypoxia is accompanied by increased binding of a 66-kDa protein (hypoxia-inducible protein, HIP) to the pyrimidine-rich tract located between bases 1552 and 1578 in the 3′-UTR of TH mRNA (18). This element has been referred to as the hypoxia-inducible protein binding site (HIPBS). The present study characterizes the sequence specificity and maps the HIP binding site within the pyrimidine-rich region of the TH mRNA 3′-UTR. We report that the motif (U/C)(C/U)CCCU within the pyrimidine-rich tract represents the optimum HIP-binding site. Importantly, the middle two cytidines (underlined) are the protein core binding site.

MATERIALS AND METHODS

The experimental procedures were described earlier (18). The restriction enzymes were purchased from Promega Corp. and Life Technologies, Inc., whereas the chemicals were purchased from Fisher and Sigma.
RNA Constructs—Transcription plasmid that contains wild type fragment KpnI–SphI (bases 1521–1682) of TH cDNA (pSP73-TH162 (18)) was used to obtain six different mutations of HIPBS. First, two new unique restriction sites, NcoI and BamHI, were generated immediately 5’ and 3’ from HIPBS using recombinant polymerase chain reaction (19) with two sets of primers. An NcoI site was generated by A → T and A → G substitutions in positions 1549 and 1550, respectively, of TH cDNA, and a BamHI site was generated by insertion of an A and a C → T substitution in position 1551 of TH cDNA (see Fig. 1). The resulting plasmid is called pSP73-TH162M. Mutated plasmids were constructed by inserting synthetic, annealed oligodeoxynucleotides into which the HIPBS sequence had been mutated into an NcoI–BamHI restricted pSP73-TH162 transcription vector. All the mutants were sequenced, and their sequences are shown in Table I. For in vitro transcription, plasmids were linearized with HindIII, BamHI, or NcoI (see Fig. 1). As described above, 10% acrylamide gel was used in competition experiments. Transcribed RNAs were purified on RNase-free G-50 column (Boehringer Mannheim).

In the second series of experiments, synthetic RNA oligodeoxynucleotides were used. RNA oligodeoxynucleotides were synthesized on a nucleic acid synthesizer (Applied Biosystems model 380B) using phosphoramidites, support columns, and a deprotection protocol from Glen Research. All RNA oligodeoxynucleotides were used on 10% acrylamide gel to determine the presence of a single band. For RNA gel shift assays, RNA oligodeoxynucleotides were labeled at the 5’ end using antisense oligodeoxyribonucleotides and RNase H endogenously present in the protein extracts. Mutated RNA-protein bands were compared with the wild type RNA-protein complex obtained in each experiment (100%) and presented as the percentage of change from this control.

Mapping with Antisense Oligodeoxynucleotides and RNase H—The synthetic antisense oligodeoxynucleotides that were complementary to the sequences within HIPBS and immediate 5’- and 3’-flanking regions were used. The sequences within the TH 3’-UTR are indicated with arrows in Fig. 2A. The antisense oligodeoxynucleotides (100 ng) were annealed to the labeled RNA transcript in the binding buffer for 15 min. The protein extracts were then added, and the binding reactions were continued for 15 min. This was followed by addition of RNase T1 and heparin (as described in Refs. 18 and 20).

RESULTS

Fig. 1A shows the DNA sequence corresponding to the KpnI–SphI fragment of the 3′-UTR of TH mRNA that was used to generate transcripts for gel retardation assays. The location and sequence modification created by generation of BamHI and NcoI restriction sites are also shown. Fig. 1B shows the binding pattern of the protein to this fragment of TH mRNA 3′-UTR when the wild type DNA template (pSP73-TH162) was linearized with HindIII (full-length transcript, lane 1) and when the pSP73-TH162M template was digested with either BamHI (transcript ends immediately 3’ from HIPBS, lane 2) or NcoI (transcript ends immediately 5’ from HIPBS, lane 3). It is clear that the RNA fragment that contains HIPBS but no additional 3′ sequence binds HIP in the same manner and to the same extent as the full-length wild type transcript (compare lanes 2 and 1). However, RNA that did not include HIPBS did not bind protein (lane 3).

In order to map the protein binding site within HIPBS, we have performed selective cleavages of this sequence from either the 5′ or 3′ end using antisense oligodeoxynucleotides and RNase H endogenously present in the protein extracts. Fig. 2A shows the HIPBS including some of the 5′- and 3′-flanking sequence. The HIPBS contains a stretch of 11 uninterrupted pyrimidines (Fig. 2A, i), and our initial experiments were oriented to determine if this element is directly involved in the protein binding. The arrows (Fig. 2A, a–i) indicate the fragments of RNA that were selectively cleaved by hybridization with individual complementary antisense oligodeoxynucleotides. Fig. 2B shows gel retardation assays using RNA transcripts that have progressive deletions of HIPBS from the 5′ end (Fig. 2B, left) and from the 3′ end (Fig. 2B, right). It is clear that cleavage of the flanking sequence 5′ (Fig. 2B, lane 2) or 3′ (Fig. 2B, lane 7) outside of HIPBS did not affect the protein binding. The cleavage within HIPBS from the 5′ end somewhat

### Table I

| RNA | HIP-binding sequence | Mean ± S.E. n |
|-----|----------------------|--------------|
| Wild type | UCUCCA UCCCCU UCCCCACCUACCUCU | 177 ± 14.6 3 |
| C6,18,19 | UCUCCA UCCCCU UCCCCACCUACCUCU | 122 ± 6.1 3 |
| A1,2,3 | aaaaCA UCCCCU UCCCCACCUACCUCU | 41.2 ± 2.5 6 |
| A10,11,12 | UCUCCA UCCCCU UCCCCACCUACCUCU | 3.1 ± 1.2 8 |
| A13,14,15 | UCUCCA UCCCCU aaaaCAACCUCUACCUCU | 42.2 ± 10.8 7 |
| A17,18,19 | UCUCCA UCCCCU UCCCCACCUACCUCU | 29 ± 5.3 6 |

**Fig. 1.** A, sequence of the 162-base pair, KpnI–SphI fragment of TH mRNA template that was used to generate different transcripts (plasmids pSP73-TH162 and pSP73-TH162M). The pyrimidine-rich sequence that encodes HIPBS is boxed. The naturally occurring restriction sites are indicated. The newly generated restriction sites, NcoI and BamHI, are marked by asterisks. B, gel retardation assays using S-100 protein extract and transcripts that were obtained after linearization of templates with HindIII (lane 1), BamHI (lane 2), and NcoI (lane 3). The formation of the complex was the same when HindIII or BamHI linearized templates were used, but the complex was not formed when the template was linearized with NcoI.
reduced the binding (Fig. 2B, lanes 3 and 4), but only cleavages within the stretch of 11 pyrimidines prevented formation of the normally observed complex (Fig. 2B, lane 5). Similarly, cleavage from the 3' end revealed that although binding was decreased by consequent shortening of HIPBS, it was not abolished until the deletion occurred within the stretch of 11 pyrimidines (Fig. 2B, compare lanes 8–10). In addition, the antisense oligodeoxynucleotide that was complementary to this stretch of 11 pyrimidines was able to abolish the binding (lane 11). Moreover, in all cases where the formation of RNA-protein complexes was only reduced but not abolished, the binding was enhanced when protein extracts were obtained from hypoxic cells (not shown).

We next examined the effects of mutations within the pyrimidine tract on binding of the HIP using RNA gel shift assays with the S-100 fraction of cytoplasmic proteins from PC12 cells. Table I shows sequences of HIPBS mutations that were introduced using the Ncol–BamHl restriction frame. Fig. 3 shows the binding pattern of HIP to the mutated HIPBS. The quantification of average data from several such experiments is presented in Table I.

First we tested whether increasing the pyrimidine content of HIPBS sequence would affect the binding. Interestingly, substitution of cytidines for three naturally occurring adenines, mutation C6,18,19, that created additional stretches of oligo(C) in HIPBS, increased the binding by approximately 80% (Fig. 3, lane 2). Substitution of adenines by the other pyrimidine nucleotide, uridine, mutation U6,18,19, was less effective than the above mutation and only increased the binding by approximately 20% as compared with the wild type control (Fig. 3, lane 3).

We have also tested whether decreasing the pyrimidine content of HIPBS and in particular whether interrupting the stretch of 11 pyrimidines affects the HIP binding properties. Mutation A10,11,12, which disrupted the stretch of 11 pyrimidines and the oligo(C) sequence, completely abolished the binding (Fig. 3, lane 6). In contrast, the mutation A13,14,15, located immediately 3' to A10,11,12, which also disrupted the stretch of 11 pyrimidines but without affecting the UCCCU element, did not abolish the binding, but only reduced it (Fig. 3, compare lanes 6 and 7). The other mutations, A1,2,3, and A22,24, in which adenines were substituted for pyrimidines outside the 11-pyrimidine stretch and which did not disrupt the UCCCU element, reduced the HIP binding to about 30–40%. The affinity of the mutated HIP-binding sequences for HIP was further tested using competition experiments in which the binding of HIP to the wild type transcript was competed with the indicated doses of unlabeled, mutated RNAs in the RNA gel shift assays. Fig. 4 shows the results of these experiments. The binding was reduced by approximately equal doses of cold wild type and mutant U6,18,19 RNAs. The dose of the cold RNAs that completely abolished the binding was somewhat above 100 ng, because at 100 ng of cold competitor some binding was still visible in both (Fig. 3, lanes 2–4 and 8–10). The binding was competed more strongly with the C6,18,19 RNA that also showed stronger binding affinity in the gel shift assays. The competing dose was below 100 ng (Fig. 3, lanes 5–7). The mutant A10,11,12 RNA that did not show any binding activity in RNA gel shift assays did not compete for binding at doses of cold probe up to 500 ng (Fig. 3, lanes 14–16). The three other mutant RNAs, A1,2,3, A13,14,15, and A22,24, showed intermediate strength in the competition experiments that paralleled their HIP binding affinities.

Finally, we tested whether the HIPBS-mutated RNAs maintained the ability to bind cytoplasmic protein in the hypoxia-inducible manner. Fig. 5 shows that except for mutant A10,11,12, which does not bind protein (Fig. 5, lanes 9 and 10), all other mutant RNAs were capable of forming increased RNA-protein complexes when S-100 protein extracts were obtained from...
Fig. 4. Competition of TH mRNA-protein binding with wild type and mutated (mutated HIPBS) cold RNAs at the indicated concentrations. Mutation A7-A10, A11, A12 did not compete for the binding (lanes 14–16), and mutation C9, C10, C11 was the strongest competitor (lanes 5–7). The mutations that moderately increased (lanes 8–10) or partially decreased (lanes 11–13 and 17–22) the binding reduced the complex formation parallel to their binding affinities.

Fig. 5. Effects of hypoxia on the formation of mutated TH mRNA-protein complex. In all cases, except when mutation abolished the binding, the formation of the complex was increased when protein extracts were obtained from PC12 cells exposed to 5% O2 for 3 h compared with the extracts obtained from cells grown in normoxia (21% O2). How-ever, the hypoxia-induced increase in the complex formation was proportional to the binding affinity with normoxic protein extracts. The results of the above experiments indicate that the core binding site for the hypoxia-inducible protein is within the UCCCU element and is represented by the stretch of four cytidines.

We next mapped the protein binding site within the UCCCU element using mutated synthetic RNA oligoribonucleotides. Fig. 6 shows the binding features of the hypoxia-inducible protein to the synthetic 27-base RNA oligonucleotide that has the HIPBS sequence. It is clear that the binding complex between protein and the HIPBS RNA oligonucleotide (Fig. 6A, lanes 3 and 4) migrates at the same level as the complex formed between the same amount of protein extract and the wild type (162 TH) RNA transcript (Fig. 6A, lane 1). RNA oligonucleotide that has a random sequence does not form a complex with the proteins (Fig. 6A, lanes 6 and 7). In addition, the formation of the complex between the 27-base HIPBS oligoribonucleotide and the protein is increased when protein extracts are obtained from PC12 cells exposed to hypoxia (Fig. 6A, H, lane 4) as compared with extracts of cells exposed to 21% O2 (Fig. 6A, C, lane 3). Moreover, we showed that the HIPBS RNA oligonucleotide is capable of competing for the HIP with the full-length 162-base RNA transcript, because the addition of 100 ng of HIPBS abolishes the formation of the RNA-HIP complex (Fig. 6B, lanes 2–4). In contrast, the random 27-base RNA oligonucleotide that does not bind protein (Fig. 6A, lanes 5–7) does not abolish the binding (Fig. 6B, lanes 5–7).

Table II lists sequences of the synthetic RNA oligonucleotides that were tested. Fig. 7A shows RNA gel shift assays with these RNA oligonucleotides. Fig. 7B shows competition experiments in which the wild type and each mutated synthetic oligoribonucleotide (100 ng) were tested for their ability to reduce or abolish formation of the complex between HIP and the wild type 162-base fragment of TH mRNA (162tr). Quantification of the average data is included in Table II.

Point substitutions of adenines for each of the bases within the UCCCU element were detrimental to binding below 50% of the binding with the 162 transcript. The binding reduction was extreme (to ~10%) when either of the two middle cytidines was substituted by an adenine (A9 or A10; Fig. 7A, lanes 4 and 5). These two mutants did not compete with the wild transcript for protein binding (Fig. 7B, lanes 5 and 6). Interestingly, substitution with the other pyrimidine, uridine, for C9 or C10 also strongly reduced the binding to approximately 20% (U9 and U10; Fig. 7A, lanes 10 and 11). These mutant HIPBS were also weak competitors, although stronger than A9 and A10 (Fig. 7B, lanes 12 and 13). The binding was partially reduced (30–40%) when U7, C8, C11, or U12 were substituted by an adenine (A7, A8, A11, or A12; Fig. 7A, lanes 2, 3, 5, and 6). These mutants also had the ability to compete with 162tr for protein binding in proportion to the complex formation (Fig. 7B, lanes 3, 4, 7, and 8). Substitution of both external cytidines by adenines, creating the sequence UACCAU, completely abolished the binding (not shown).

Interestingly, U → C substitutions in position 8 (U9) (UUC-
CCU) substantially increased the binding to 163.6% (Fig. 7A, lane 9). This mutant was also a strong competitor of the wild type HIPBS RNA oligonucleotide (Fig. 7B, lane 11). In contrast, U substitutions in position 11 (U11) (UCCC/UU) decreased the binding to 55.6% (Fig. 7A, lane 12). Substitution of both external cytidines (C8 and C11) by uridine, which created the sequence UUCCUU, completely abolished binding (not shown), similar to corresponding substitutions with adenines. In addition, insertions of an individual adenine between the cytidines in the UCCCCU motif, creating the sequence UCCACCU or UCCCCAU, reduced the binding below 20% (not shown). Interestingly, the substitutions C → U (C7, lane 14) and C → U12 (C12, lane 15) had the opposite effects on the formation of RNA-protein complexes. The C7 mutation caused nearly a 2-fold increase in binding, whereas the C12 mutation decreased binding by 30%. Both substitutions together (C7,12) had an additive effect such that the total increase in binding was less pronounced than in the case of the C7 mutation alone (lane 16). In spite of the fact that the C7 mutant increased binding, its ability to compete with the 162tr for protein binding was slightly decreased compared with that of the wild type HIPBS RNA oligonucleotide (Fig. 7B, lane 17).

None of the point mutations in the HIPBS sequence outside the UCCCCU element substantially affected binding. On the other hand, mutation of the pyrimidine-rich region into the random sequence on either or both sides of the UCCCCU element completely abolished binding (not shown).

**DISCUSSION**

We have previously shown that the pyrimidine-rich region (HIPBS) in the 3'-untranslated region of tyrosine hydroxylase mRNA binds a 66-kDa protein in a hypoxia-inducible manner (18). Those results suggested potential involvement of HIPBS in the regulation of TH mRNA stability during hypoxia (15). In this study we have shown that under in vitro conditions, the

**TABLE II**

Nucleotide substitutions within HIPBS that were obtained using synthetic oligoribonucleotides

Average data represent the percentage of change in the protein-mutated RNA oligonucleotide complex formation as compared with the complex formed between the protein-wild type RNA oligonucleotide in the same experiment (100%). Data were normalized to the total amount of radioactivity loaded in each lane. n, number of experiments.

| Mutations | HIP-binding sequence | Mean ± S.E. | n |
|-----------|----------------------|-------------|---|
| Wild type | UCUCCA UCCCCU UCCCCACCUUUCCU | | |
| A substitutions | | | |
| A7 | UCUCCA aCCCCU UCCCCACCUUUCCU | 41.4 ± 6.2 | 7 |
| A8 | UCUCCA UCCCCU UCCCCACCUUUCCU | 25 ± 7.5 | 5 |
| A9 | UCUCCA UCCCCU UCCCCACCUUUCCU | 10.3 ± 3.2 | 6 |
| A10 | UCUCCA UCCCCU UCCCCACCUUUCCU | 11.8 ± 3.1 | 8 |
| A11 | UCUCCA UCCCCU UCCCCACCUUUCCU | 39 ± 6.1 | 3 |
| A12 | UCUCCA UCCCCU UCCCCACCUUUCCU | 38 ± 7.5 | 6 |
| U substitutions | | | |
| U8 | UCUCCA UCCCCU UCCCCACCUUUCCU | 163 ± 12.7 | 5 |
| U9 | UCUCCA UCCCCU UCCCCACCUUUCCU | 18.5 ± 3 | 5 |
| U10 | UCUCCA UCCCCU UCCCCACCUUUCCU | 15 ± 0.18 | 9 |
| U11 | UCUCCA UCCCCU UCCCCACCUUUCCU | 55 ± 6 | 5 |
| C substitutions | | | |
| C7 | UCUCCA cCCCCU UCCCCACCUUUCCU | 186 ± 12 | 5 |
| C12 | UCUCCA UCCCCc UCCCCACCUUUCCU | 71 ± 14 | 5 |
| C7,12 | UCUCCA cCCCCc UCCCCACCUUUCCU | 161 ± 12 | 5 |
This effect was particularly strong in the case of C9 and C10, specifically for the presence of cytidine in these positions. Importantly, however, some pyrimidine substitutions are also detrimental to the binding within this motif are detrimental to the binding.

We have hypothesized that the binding site may be represented by the sequence UCCCCU with a preference for uridine rather than cytidine content (uridine) of HIPBS increased the binding, we have specifically oligo(C) but not simply an increase in pyrimidine content the oligo(C) tract and increased the formation of RNA-protein complexes, the mutation did not result in an increased ability to compete with the wild type RNA transcript for the protein binding. There is, therefore, preference for the UCCCCU sequence rather than the oligo(C) stretch as the optimal binding site for HIP.

It is important to note that not all oligo(C) stretches are capable of binding HIP protein. For example a 21-base-long sequence within the 3′-UTR of TH mRNA that is relatively rich (although less so then HIPBS) in pyrimidines and has an ACCCCCA sequence at the beginning of the poly(Y) tract (Table III) does not bind HIP (18). Thus, the optimal HIP binding element is represented by the sequence UCCCCU with the similar binding properties for sequence UUCCCU and may be summarized by the consensus (U/C)(C/U)CCCnU. In addition, each of these mRNAs has an ACACCUC sequence at the beginning of the poly(Y) tract (Table III) does not bind HIP (18). Thus, the optimal HIP binding element is represented by the sequence UCCCCU with the similar binding properties for sequence UUCCCU and may be summarized by the consensus (U/C)(C/U)CCCnU.

Our results demonstrate that the major binding site for the HIP is the UCCCCU motif, as point mutations only within this element could either substantially reduce or almost abolish the complex formation, and multiple mutations within this region completely abolished the binding. In view of the facts that 1) this element has an oligo(C) stretch, 2) the protein binding is strongly competed by polycytidine RNA (18), and 3) creation of this element has an oligo(C) stretch, 2) the protein binding is completely abolished the binding. In view of the facts that 1) this element has an oligo(C) stretch, 2) the protein binding is completely abolished the binding.

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Our results demonstrate that the major binding site for the HIP is the UCCCCU motif, as point mutations only within this element could either substantially reduce or almost abol
been made to define the RNA consensus elements that preferentially bind these proteins using in vitro genetic selection (14) as well as mutational analysis of their binding properties (20, 21). In this respect two of these proteins, the poly(Y) tract-binding protein (8) and the Sxl protein (10), show preferential affinity for the polyuridine containing sequences, and their consensus binding sites are different from the HIP binding site (14). There is some degree of similarity between the HIP binding site and the consensus element that binds the U2AF65 (14). Results from the genetic selection showed that the U2AF65 binds preferentially to the sequences rich in uridines that are frequently interrupted by two to three cytidines (14). HIP, however, seems to bind preferentially to the sequences enriched in cytidines that are interrupted by one to two uridines. In view of the high affinity of HIP for oligo(C) sequences, the polycytidine-binding protein K, which is similar in molecular mass to HIP (68 kDa) (13), needs to be considered as the potential HIPBS-binding protein. The HIP consensus sequence is also homologous with the polypyrimidine motif (C4PuC3UCUUUC4AAG) located in the 3′-UTR of 15-lipoxygenase (23). However, the 15-lipoxygenase mRNA-binding protein has a molecular mass of 48 kDa, which is substantially smaller than the 66-kDa HIP.

We have also analyzed for the presence of the HIPBS-like sequences a number of 3′-UTRs from other mRNAs that are likely to be regulated by hypoxia (Table IV). We were able to identify a HIPBS-like element in the 3′-UTR of erythropoietin, inducible nitric oxide synthase, tumor necrosis factor α, myoglobin, and tryptophan hydroxylase mRNAs. Currently, the post-transcriptional regulation of the genes listed in Table IV is not well known. It has been studied best in the case of erythropoietin mRNA, to which binding of cytoplasmic protein to the HIPBS-like region was reported (24). In other cases there is only indirect evidence of this regulation.

Acknowledgments—We thank Drs. D. E. Millhorn and W. F. Marzluff for comments during preparation of the manuscript and Glenn E. Doerman for preparation of the figures.

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