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Bovine heart fructose-6-phosphate 2-kinase/fructose-2,6-bisphosphatase: Complete amino acid sequence and localization of phosphorylation sites

(fructose 2,6-bisphosphate/glycolysis)

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ABSTRACT **We have shown previously that bovine heart fructose-6-phosphate 2-kinase/fructose-2,6-bisphosphatase (EC 2.7.1.105/3.1.3.46) is phosphorylated by cAMP-dependent protein kinase and protein kinase C; phosphorylation results in activation of kinase. This activation of heart enzyme is in contrast to results with the liver isozyme, in which phosphorylation by cAMP-dependent protein kinase inhibits the kinase activity. As an initial step toward understanding this difference between the isozymes we have determined the DNA sequence of the heart enzyme and analyzed the amino acid sequence with special emphasis on the location of the phosphorylation site. We isolated and sequenced two overlapping cDNA fragments, which together could encode the complete amino acid sequence of bovine heart fructose-6-phosphate 2-kinase/fructose-2,6-bisphosphatase, a protein of 530 amino acids, with a calculated molecular weight of 60,679. Since the deduced protein contained amino acid sequences identical to the sequences of four known tryptic peptides from this enzyme we concluded that the deduced protein sequence did represent bovine heart enzyme. In addition, a cDNA fragment hybridized to a 4-kilobase mRNA from bovine heart. The phosphorylation sites of the heart enzyme were located near the C terminus, whereas the phosphorylation site of the liver isozyme is known to be located near the N terminus. These opposite locations of the phosphorylation sites may explain the contrasting effect of the covalent modification on the enzymes' activities.**

The synthesis and degradation of fructose 2,6-bisphosphate (Fru-2,6-P_2) are catalyzed by a bifunctional enzyme, fructose-6-phosphate (Fru-6-P) 2-kinase/fructose-2,6-bisphosphatase (Fru-2,6-P_2ase) (EC 2.7.1.105/3.1.3.46: Fru-6-P + ATP $\rightleftharpoons$ Fru-2,6-P_2 + ADP and Fru-2,6-$P_2 \rightarrow$ Fru-6-P + P_i) (1–6). Three major isozymic forms, namely the liver, muscle, and heart types, occur in various mammalian tissues. They differ in their molecular weights, immunoreactivity, and kinetic properties (7–11). Perhaps the most interesting difference between the liver and the heart isozymes is the regulation of enzyme activities by phosphorylation catalyzed by cAMP-dependent protein kinase and protein kinase C. When the liver enzyme is phosphorylated by cAMP-dependent protein kinase it results in inhibition of the kinase activity and activation of the phosphatase activity (12–14). This reciprocal change in the kinase and the phosphatase activities of the bifunctional enzyme explains the action of glucagon in decreasing Fru-2,6-P_2 concentration in liver, which causes the inhibition of phosphofructokinase and glycolysis. We have shown (15) that the heart Fru-6-P 2-kinase/Fru-2,6-P_2ase is also phosphorylated by cAMP-dependent protein kinase, but in contrast to the liver isozyme, the heart kinase is activated by the covalent modification. This observation accounts for the increased Fru-2,6-P_2 in perfused heart as a result of epinephrine administration (16). Protein kinase C also phosphorylates the heart enzyme, resulting in activation (17). That protein kinase A-catalyzed phosphorylation has opposite effects on the two isozymes is, to our knowledge, unique to Fru-6-P 2-kinase/Fru-2,6-P_2ase. In all other cases where phosphorylation changes the activities of those isozymes, they are altered in the same direction—i.e., activation or inhibition, regardless of the tissue origin. Therefore, it is of considerable interest to understand the biochemical mechanism for this opposite effect on the liver and the heart Fru-6-P 2-kinase/Fru-2,6-P_2ase. One simple explanation is a difference in their Fru-6-P binding sites, since the K_m for this substrate was altered in the opposite direction by the phosphorylation. This explanation is not likely, however, since we demonstrated that the amino acid sequences around the Fru-6-P binding sites are similar (18). As far as we are aware, the isozymes from different tissues are altered in the same direction by phosphorylation, and also in all these cases the phosphorylation sites are located in the same terminus of the enzymes. Therefore, another possibility considered is that the phosphorylation sites of these isozymes are at the opposite ends of the molecules. The phosphorylation site of rat liver Fru-6-P 2-kinase/Fru-2,6-P_2 is at Ser-32 near the N terminus (19). The sites of bovine heart Fru-6-P 2-kinase/Fru-2,6-P_2ase phosphorylated by cAMP-dependent protein kinase and protein kinase C are located near each other (17), but their exact locations in the enzyme molecule were not determined.

In this communication we describe the isolation and determination of the DNA sequence of bovine heart Fru-6-P 2-kinase/Fru-2,6-P_2ase.† On the basis of the deduced amino acid sequence, we determined that the phosphorylation sites in the heart isozyme are localized near the C terminus, opposite from the site of the liver isozyme.

MATERIALS AND METHODS

Materials. The λgt11 bovine heart cDNA library was purchased from Clontech. The cDNA probe encoding human liver Fru-6-P 2-kinase/Fru-2,6-P_2ase was prepared as described (20). Other materials were purchased from commercial sources.

Isolation and DNA Sequence Determination of Bovine Heart Fru-6-P 2-Kinase/Fru-2,6-P_2ase Clones. The cDNA encoding

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Abbreviations: Fru-2,6-P_2, fructose 2,6-bisphosphate; Fru-6-P, fructose 6-phosphate; Fru-2,6-P_2ase, fructose-2,6-bisphosphatase.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34241).

human liver Fru-6-P 2-kinase/Fru-2,6-P_2ase was used initially as a probe to screen the λgt11 bovine heart cDNA library, and a strongly positive DNA fragment of a 1.4 kilobases (kb) was used to rescreen the same library. Growth of phages on agar plates, transfer of the phage plaques on filters, phage lysis, and DNA covalent binding were as described by Maniatis *et al.* (21). These filters were prehybridized for 4–8 hr at 65°C in 1% sodium dodecyl sulfate/1 M NaCl containing fish DNA at 100 μg/ml and hybridized in the same solution containing a ^{32}P-labeled probe at the same temperature overnight. The probes were radiolabeled with [α-^{32}P]dCTP by using a random oligo-labeling kit (Pharmacia). The filters were washed several times with 0.3 M NaCl/30 mM sodium citrate/0.2% sodium dodecyl sulfate at 60°C. The filters were partially dried and subjected to autoradiography. For sequencing the DNA inserts were isolated and subcloned in bacteriophage M13 vectors (22) and sequenced by dideoxynucleotide method (23) using various oligonucleotides as primers.

RNA Blot Hybridization Analysis. Poly(A)$^+$ RNA samples were prepared from frozen bovine tissues by using an RNA isolation kit (Invitrogen, San Diego). Those tissues were obtained from a local slaughterhouse, frozen in liquid nitrogen immediately, and stored at −70°C. The RNAs were electrophoresed through a formaldehyde/agarose gel (21), transferred to a Zeta-Probe filter (Bio-Rad), and hybridized with the random-prime labeled bovine heart cDNA as described above.

RESULTS

Isolation of Heart Fru-6-P 2-Kinase/Fru-2,6-P_2ase cDNA Clones and Determination of Nucleotide Sequences. Initial screening of the bovine heart cDNA library with a human liver cDNA probe (20) yielded a positive λgt11 clone containing a 1.4-kb insert. This insert, designated as BH7H, was too small to encode the whole enzyme, so it was purified and used as a probe to rescreen the bovine heart library. This rescreening produced another positive λgt11 clone containing a 1.0-kb insert, designated as BH9G. The fragments BH7H and BH9G were cloned in M13, and their DNA sequences were determined as described in *Materials and Methods*. The restriction map and DNA sequence results (see below) showed that the fragments overlapped as diagrammed in Fig. 1. Fig. 2 shows the composite nucleotide sequence of the DNA carried by these two fragments. The 3′ end of BH9G (from nucleotide 468 to nucleotide 971 in Fig. 2) overlapped with the first 504 nucleotides of the 5′ end of BH7H. The BH9G fragment contained 26 nucleotides of 5′ untranslated sequence followed by an initiation codon. This initiation site was favored over two other sites, which are approximately 100 and 130 nucleotides downstream, because the surrounding nucleotide sequence has the general consensus structure of a eukaryotic initiation site (24). Counting from this initiation site, the fragment had 942 nucleotides of open reading frame, capable of encoding a peptide of 314 amino acids.

The BH7H fragment contained the next 1152 nucleotides of open reading frame and could encode a peptide of 384 amino acids. The 3′ untranslated sequence which followed the TGA termination codon at 1620 was 213 nucleotides.

Amino Acid Sequence. Fig. 2 also shows the deduced amino acid sequence of this open reading frame. The previously determined peptide sequences of the four CNBr or tryptic peptides of Fru-6-P 2-kinase/Fru-2,6-P_2ase (17, 18) were found in the predicted amino acid sequence determined from these fragments (Fig. 2, underlined sequences). These results supported the conclusion that these two overlapping fragments encoded the entire bovine heart Fru-6-P 2-kinase/Fru-2,6-P_2ase. Using the above start site, the combined DNA would encode a protein of 530 amino acids with a calculated M_r of 60,679.

Amino Acid Sequence Comparisons. Fig. 3 shows a comparison of the amino acid sequence of bovine heart Fru-6-P 2-kinase/Fru-2,6-P_2ase with the published sequence of rat liver Fru-6-P 2-kinase/Fru-2,6-P_2ase (19, 25). To maximize the alignment of the two sequences, two single amino acid gaps had to be added to the rat liver enzyme sequence. The comparison revealed several interesting features. These two sequences were approximately 66% identical, and the major differences occurred mostly in the N and C termini of these proteins. The heart enzyme sequence extend 61 amino acid residues past the C terminus of the liver enzyme sequence. This C-terminal peptide of the heart enzyme contained the phosphorylation sites (boxed in Fig. 3) for both cAMP-dependent protein kinase and protein kinase C (17). In contrast, the phosphorylation site for cAMP-dependent protein kinase of the liver isozyme (boxed in Fig. 3) is known to be in the N-terminal region (19). There are a number of peptides whose amino acid sequences are well conserved in these two isozymes. Among the identical peptides are the "signature" sequence for the nucleotide binding domain (26) at Gly-Leu-Pro-Ala-Arg-Gly-Lys-Thr (residues 45–52) of the heart enzyme and the Fru-6-P and Fru-2,6-P_2 binding sites of Fru-6-P 2-kinase (18, 27) at Lys-Gln-Cys-Ala-Leu-Val-Ala-Leu-Lys-Asp-Val-Lys-Ala-Tyr (residues 103–116), Arg-Ile-Glu-Cys-Tyr-Lys (residues 193–198), and Asp-Glu-Glu-Lys-Tyr-Leu (residues 352–357) of the heart enzyme. In addition, the liver bisphosphatase active site (28) is known to contain Arg-His-Gly and is conserved in the heart enzyme at Arg-His-Gly (residues 256–258).

mRNA. Bovine heart poly(A)$^+$ RNA contained a 4-kb hybridizing species (Fig. 4), but total RNA preparations did not show any detectable hybridization band, indicating low abundance of the mRNA.

DISCUSSION

In this report the isolation and characterization of cDNA clones encoding the complete sequence of bovine heart

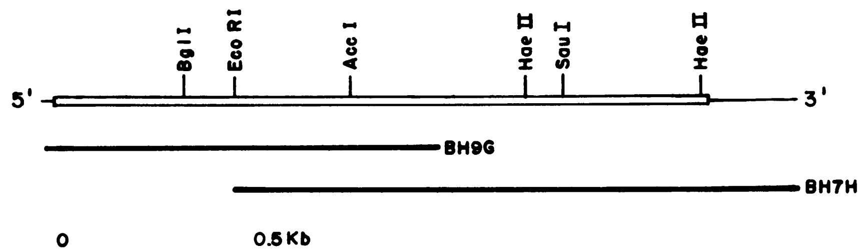

FIG. 1. Schematic representation of the two overlapping fragments containing bovine heart Fru-6-P 2-kinase/Fru-2,6-P_2ase cDNA. The bar indicates the coding region; lines under the bar show the DNA contained in the fragments BH9G and BH7H. Restriction sites are indicated on the bar.

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   1 caaccgacatctcctgaagagttgccatgTCCGGGAATCCTGCCTCTTCCTCAGAACAGAAC
                                  SerGlyAsnProAlaSerSerSerGluGlnAsn  11

  63 AACAATAGCTATGAAACCAAAGCAAGTCTCCGAATATCAGAGAAGAAATGTTCATGGGCA
     AsnAsnSerTyrGluThrLysAlaSerLeuArgIleSerGluLysLysCysSerTrpAla   31

 123 TCTTACATGACAAACTCTCCAACCCTCATCGTTATGATTGGCTTGCCAGCCCGTGGCAAA
     SerTyrMETThrAsnSerProThrLeuIleValMETIleGlyLeuProAlaArgGlyLys   51

 183 ACCTACGTGTCCAAGAAACTTACACGCTACCTCAACTGGATTGGGGTGCCCACCAAAGTG
     ThrTyrValSerLysLysLeuThrArgTyrLeuAsnTrpIleGlyValProThrLysVal   71

 243 TTTAATCTTGGGGTGTATCGGCGGCAAGCAGTCAAGTCCTATAAGTCCTATGACTTCTTC
     PheAsnLeuGlyValTyrArgArgGlnAlaValLysSerTyrLysSerTyrAspPhePhe   91

 303 CGGCATGACAACGAGGAGGCCATGAAGATTCGCAAACAGTGTGCCCTGGTGGCACTGAAA
     ArgHisAspAsnGluGluAlaMETLysIleArgLysGlnCysAlaLeuValAlaLeuLys  111

 363 GACGTGAAGGCGTATCTCACGGAGGAGAGCGGGCAGATTGCGGTGTTTGATGCTACCAAT
     AspValLysAlaTyrLeuThrGluGluSerGlyGlnIleAlaValPheAspAlaThrAsn  131

 423 ACCACTCGAGAGAGGAGGGACTTGATTTTGAACTTTGCCGAGGAGAATTCTTTCAAGGTG
     ThrThrArgGluArgArgAspLeuIleLeuAsnPheAlaGluGluAsnSerPheLysVal  151

 483 TTCTTTGTGGAGTCCGTGTGCGATGATCCTGATGTCATTGCTGCCAACATCCTGGAGGTA
     PhePheValGluSerValCysAspAspProAspValIleAlaAlaAsnIleLeuGluVal  171

 543 AAGGTGTCGAGCCCCGACTACCCTGAAAGGAACAGGGAGAATGTGATGGATGACTTCCTA
     LysValSerSerProAspTyrProGluArgAsnArgGluAsnValMETAspAspPheLeu  191

 603 AAGAGGATTGAGTGCTACAAAGTCACCTATCAACCCCTTGACCCAGACAGCCATGACAAG
     LysArgIleGluCysTyrLysValThrTyrGlnProLeuAspProAspSerHisAspLys  211

 663 GATCTTTCTTTCATCAAGGTGATAAACGTGGGCCAGAGATTTCTCGTGAACAAAGTCCAG
     AspLeuSerPheIleLysValIleAsnValGlyGlnArgPheLeuValAsnLysValGln  231

 723 GACTACATCCAGAGCAAGATCGTCTACTACCTCATGAATATCCACGTCCACCCTCGCACC
     AspTyrIleGlnSerLysIleValTyrTyrLeuMETAsnIleHisValHisProArgThr  251

 783 ATCTACCTTTGCCGGCATGGAGAGAGCGAGTTCAACCTCTTGGGGAAGATTGGGGGTGAC
     IleTyrLeuCysArgHisGlyGluSerGluPheAsnLeuLeuGlyLysIleGlyGlyAsp  271

 843 TCAGGCCTCTCAGTGCGAGGAAAACAGTTTGCCCAGGCTCTAAGGAAGTTTCTGGAGGAA
     SerGlyLeuSerValArgGlyLysGlnPheAlaGlnAlaLeuArgLysPheLeuGluGlu  291

 903 CAGGAGATAGCAGACCTCAAAGTGTGGACGAGCCAGTTGAAAAGGACTATCCAGACCGCA
     GlnGluIleAlaAspLeuLysValTrpThrSerGlnLeuLysArgThrIleGlnThrAla  311

 963 GAATCTCTGGGGGTGACCTACGAGCAGTGGAAGATTCTGAACGAGATTGATGCTGGCGTG
     GluSerLeuGlyValThrTyrGluGlnTrpLysIleLeuAsnGluIleAspAlaGlyVal  331

1023 TGCGAGGAGATGACTTACGCGGAGATTCAGGAGCAGTATCCGGATGAGTTTGCGCTTCGA
     CysGluGluMETThrTyrAlaGluIleGlnGluGlnTyrProAspGluPheAlaLeuArg  351

1083 GATGAAGAGAAATATCTGTACCGATATCCTGGAGGGGAGTCATACCAGGACCTCGTGCAG
     AspGluGluLysTyrLeuTyrArgTyrProGlyGlyGluSerTyrGlnAspLeuValGln  371

1143 CGGTTGGAGCCAGTCATCATGGAGCTGGAGCGCCAGGGCAATGTCCTCGTTATCTCCCAC
     ArgLeuGluProValIleMETGluLeuGluArgGlnGlyAsnValLeuValIleSerHis  391

1203 CAGGCTGTCATGCGCTGCCTCCTGGCCTACTTCTTGGACAAGGGTGCAGATGAGCTACCA
     GlnAlaValMETArgCysLeuLeuAlaTyrPheLeuAspLysGlyAlaAspGluLeuPro  411

1263 TACCTGAGGTGCCCTCTCCATACCATCTTCAAACTTACTCCTGTGGCCTACGGGTGCAAA
     TyrLeuArgCysProLeuHisThrIlePheLysLeuThrProValAlaTyrGlyCysLys  431

1323 GTGGAAACAATTAAACTCAATGTGGAGGCTGTGAACACGCACCGTGACAAGCCAACTAAC
     ValGluThrIleLysLeuAsnValGluAlaValAsnThrHisArgAspLysProThrAsn  451

1383 AACTTTCCCAAGAGCCAAACCCCTGTAAGGATGAGAAGGAACAGCTTTACGCCTCTGTCC
     AsnPheProLysSerGlnThrProValArgMETArgArgAsnSerPheThrProLeuSer  471

1443 AGTTCGAATACAATCAGGCGTCCAAGAAATTACAGTGTTGGGAGCCGGCCCCTCCAGCCC
     SerSerAsnThrIleArgArgProArgAsnTyrSerValGlySerArgProLeuGlnPro  491

1503 CTCAGCCCGCTCCGTGCCCTGGACACGCAAGAAGGGGCCGACCAGCCGAAGACCCAAGCA
     LeuSerProLeuArgAlaLeuAspThrGlnGluGlyAlaAspGlnProLysThrGlnAla  511

1563 GAGACCTCGCGGGCTGCACACAGGCTCCCGTCTCCAGCGCCCCCCACGTCGCCCTCCtga
     GluThrSerArgAlaAlaHisArgLeuProSerProAlaProProThrSerProSer---  530

1623 tggcgtggaggctgaggccagaacgctcccccggagactggggtctgctgaagacctggg
1683 cgtgtcagctccactggggctggagcaggaagtcccgtggggctcggacattcggggcca
1743 cccgagtgagcctggaaaagctgccctccatttcttcccctcatgcctaatgaaaaccgt
1803 gaaaagctcggcttccctgtacctggcccctcg
```

FIG. 2. Nucleotide and predicted amino acid sequence. The nucleotide sequence begins with the 5′ end of the DNA insert; nucleotides representing the proposed coding region are capitalized. Numbers on the left refer to the nucleotide sequence. The amino acid sequence given in three-letter code below the nucleotide sequence is numbered on the right. The known sequences of CNBr or tryptic peptides are underlined.

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                  10        20        30        40
Heart      SGNPASSSEQNNNSYETKASLRISEKKCSWASYMTNSPTLIVMIGLP
Liver   SREM-ELTQTRL-KIWIPHSSS-SVLQRRRGSSIPQF-----MVI-V---

        50        60        70        80        90
Heart   ARGKTYVSKKLTRYLNWIGVPTKVFNLGVYRRQAVKSYKSYDFFRHDNEE
Liver   ------I-T----------T--------Q---*EAV--RN-E---P--T-

        100       110       120       130       140
Heart   AMKIRKQCALVALKDVKAYLTEESGQIAVFDATNTTRERRDLILNFAEEN
Liver   -QL-------A-----HK--SR-E-HV------------S---Q--K-H

        150       160       170       180       190
Heart   SFKVFFVESVCDDPDVIAANILEVKVSSPDYPERNRENVMDDFLKRIECY
Liver   GY----I--I-N--EI--E--KQ--LG----IDCDQ-K-LE---------

        200       210       220       230       240
Heart   KVTYQPLDPDSHDKDLSFIKVINVGQRFLVNKVQDYIQSKIVYYLMNIHV
Liver   EIN-----*EEL-SH--Y--IFD--T-YM--R---HV--RTA--------

        250       260       270       280       290
Heart   HPRTIYLCRHGESEFNLLGKIGGDSGLSVRGKQFAQALRKFLEEQEIADL
Liver   T--S----------L--R-R--------A----Y-Y--AN-IRS-G-SS-

        300       310       320       330       340
Heart   KVWTSQLKRTIQTAESLGVTYEQWKILNEIDAGVCEEMTYAEIQEQYPDE
Liver   -----HM--------A---P-----A--------------E----H--E-

        350       360       370       380       390
Heart   FALRDEEKYLYRYPGGESYQDLVQRLEPVIMELERQGNVLVISHQAVMRC
Liver   -----QD--R----K----E----------------E-----C-------

        400       410       420       430       440
Heart   LLAYFLDKGADELPYLRCPLHTIFKLTPVAYGCKVETIKLNVEAVNTHRD
Liver   --------SS------K-----VL---------R--S-Y-----------

        450       460       470       480       490
Heart   KPTNNFPKSQTPVRMRRNSFTPLSSSNTIRRPRNYSVGSRPLQPLSPLRA
Liver   --E-VDITREAEEALDTVPAHY

        500       510       520       530
Heart   LDTQEGADQPKTQAETSRAAHRLPSPAPPTSPS
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FIG. 3. Comparison of protein sequences (in one-letter code) of bovine heart (from Fig. 2) and rat liver (19, 25) Fru-6-*P* 2-kinase/Fru-2,6-P_2ase. Dashes in the liver sequence mean that the amino acid is identical to the heart amino acid; the asterisks at residues 80 and 206 represent the single amino acid gaps that were introduced to allow maximum similarity. The phosphorylation sites are boxed.

Fru-6-*P* 2-kinase/Fru-2,6-P_2ase is described. Although the N-terminal amino acid of the enzyme has not been determined, an initiation codon ATG at nucleotide 27 has the consensus sequence of eukaryotic initiator codons (24), thus suggesting that these fragments span the entire coding region of the enzyme. Furthermore, the blocked N-terminal amino acid of the heart enzyme is serine, which is the first amino acid of this sequence. The deduced amino acid sequence indicated that the enzyme consists of 530 amino acids, corresponding to an M_r of 60,679, slightly larger than the subunit M_r of 58,000 determined by sodium dodecyl sulfate/PAGE of the pure enzyme (29).

A comparison of the amino acid sequences of bovine heart and rat liver Fru-6-*P* 2-kinase/Fru-2,6-P_2ase reveals a number of homologous sequences. As expected, some of these well-conserved amino acid sequences are those peptides previously identified as the substrate binding sites or the active sites. For example, two Fru-6-*P* binding sites of both bovine heart and rat liver Fru-6-*P* 2-kinase identified previously by chemical modification studies are located in the N-terminal half of the enzyme at peptides Lys^{103}–Lys^{111} and Arg^{193}–Lys^{198} (18). Moreover, the Fru-2,6-P_2 binding sites of the kinase are at Asp^{112}–Tyr^{116} and Asp^{352}–Arg^{359} (27). One of the characteristic sequences of the nucleotide binding site (26) is Gly-Leu-Pro-Ala-Arg-Gly-Lys-Thr (residues 45–52) near the N terminus of the heart enzyme. The second characteristic peptide of the nucleotide binding domain has been postulated to occur in the rat liver enzyme at residues 121–130, Arg-Glu-Glu-Gly-His-Val-Ala-Val-Phe-Asp (30). However, the corresponding region in the bovine heart enzyme is the peptide Glu-Glu-Ser-Gly-Gln-Ile-Ala-Val-Phe-Asp (residues 119–128) which was considerably different. The phosphohistidine intermediate formed in the Fru-2,6-P_2ase reaction (28) is located at His-257 in the heart enzyme, and the surrounding sequence is identical to that of the liver enzyme.

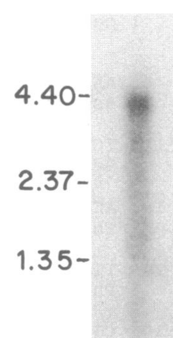

FIG. 4. Northern blot analysis of poly(A)$^+$ RNA isolated from bovine heart. Poly(A)$^+$ RNA (10 μg) from bovine heart hybridized with probe BH9G DNA. Numbers at left are lengths of markers in kb.

One of the main objectives of this investigation was to determine whether the phosphorylation sites of the heart and the liver isozymes are located on the opposite ends of the enzyme molecules, which might explain our previous observation of the activation of the heart enzyme and inhibition of the liver enzyme by the covalent modification. Previously workers in this laboratory (17) isolated and determined the amino acid sequence of a peptide containing the phosphorylation sites of the heart Fru-6-*P* 2-kinase/Fru-2,6-P_2ase by cAMP-dependent protein kinase and protein kinase C. As shown in the present work, this phosphorylated peptide was located in Arg^{463}–Pro^{479}; and Ser^{466} and Thr^{475}, respectively, are the target sites of protein kinase A and protein kinase C. More importantly, these phosphorylation sites are located near the C terminus of the heart isozyme. However, the phosphorylation site of the liver isozyme for cAMP-dependent protein kinase is located near the N terminus (19). The liver enzyme is not phosphorylated by protein kinase C. To the best of our knowledge there are no other examples of isozymes from two different tissues with phosphorylation sites located on the opposite termini. Furthermore, the C-terminal peptide of the heart Fru-6-*P* 2-kinase/Fru-2,6-P_2ase containing these phosphorylation sites was highly hydrophilic and consisted primarily of basic residues; there were eight arginine residues and two each of lysine, aspartic, and glutamic residues.

This laboratory demonstrated (15) that phosphorylation of Fru-6-*P* 2-kinase/Fru-2,6-P_2ase lowers the K_m of the heart-derived kinase for Fru-6-*P*, resulting in activation, but increases the K_m of the liver enzyme, resulting in inhibition. This unique opposite effect of phosphorylation on the Fru-6-*P* 2-kinase activities of the heart versus the liver isozymes must be due to the formation of two completely opposite conformations [R $\rightleftharpoons$ T (31)] of the enzymes. It is possible that the different conformations result from phosphorylation on the opposite termini of these two enzyme molecules. For the heart enzyme, the phosphorylated C-terminal peptide would bind to the subunit surface [as in the case of phosphorylase (32)], thus altering the structure around the Fru-6-*P* binding site and increasing the affinity for hexose 6-phosphate. In contrast, the phosphorylated N-terminal peptide of the liver enzyme would interact at different regions of the similar

subunits, altering the Fru-6-*P* binding site of the enzyme to less favorable configuration and resulting in weaker binding. Deduction of the exact biochemical mechanism explaining these differential effects of phosphorylation on Fru-6-*P* 2-kinase activities requires additional information on the three-dimensional structure and conformational states of the enzymes.

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