The C-terminal Hinge Region of Lipoic Acid-bearing Domain of E2b Is Essential for Domain Interaction with Branched-chain α-Keto Acid Dehydrogenase Kinase*

Received for publication, July 30, 2002 Published, JBC Papers in Press, August 19, 2002, DOI 10.1074/jbc.C200430200

Jacinta L. Chuang, R. Max Wynn, and David T. Chuang†

From the Departments of Biochemistry and Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas 75390

The branched-chain α-keto acid dehydrogenase (BCKD) kinase (abbreviated as BCK) down-regulates activity of the mammalian mitochondrial BCKD complex by reversible phosphorylation of the decarboxylase (E1b) component of the complex. The binding of BCK to the holotransacaylase (E2b) core of the BCKD complex results in the stimulation of BCK activity. Here we show that the lipoylated lipoic acid-bearing domain (lip-LBD) (residues 1–84) of E2b alone does not interact with BCK. However, lip-LBD constructs containing various lengths of the C-terminal hinge region of LBD are able to bind to BCK as measured by a newly developed solubility-based binding assay. Isothermal titration calorimetry measurements produced a dissociation constant of 8.06 × 10⁻⁶ M and binding enthalpy of –3.68 kcal/mol for the interaction of BCK with a construct containing lip-LBD and the Glu-Glu-Asp-Xaa-Xaa-Glu sequence of the C-terminal hinge region of LBD. These thermodynamic parameters are similar to those obtained for binding of BCK to a lipoylated di-domain construct, which harbors LBD, the entire hinge region, and the downstream subunit-binding domain of E2b. Our data establish that the C-terminal hinge region of LBD containing the above negatively charged residues is essential for the interaction between the lip-LBD construct and BCK.

The mammalian mitochondrial branched-chain α-keto acid dehydrogenase (BCKD) complex catalyzes the rate-limiting step in the oxidation of branched-chain amino acids leucine, isoleucine, and valine (1). Genetic defects in this macromolecular multi-enzyme complex result in the accumulation of branched-chain amino acids leading to inheritable maple syrup urine disease. The clinical phenotype includes early onset and often fatal acidosis, neurological derangement, and mental retardation among survivors (1). The mammalian BCKD complex is organized around a cubic core of 24-meric dihydrolipoyl transacaylase (E2b), to which multiple copies of branched-chain α-keto acid decarboxylase (E1b), dihydrolipomamide dehydrogenase (E3), BCKD kinase (abbreviated as BCK), and BCKD phosphatase are attached through ionic interactions (2–4). The activity of the BCKD complex is tightly regulated by a phosphorylation/dephosphorylation cycle in response to dietary and hormonal signals (5). Phosphorylation of Ser-292 and Ser-302 in the α subunit of E1b by BCK results in the inactivation of the BCKD complex (6).

The structures of rat BCK in the apo-, ADP-bound, and ATP/S-bound forms were recently determined (7). The BCK structures feature a nucleotide-binding (K) domain and a four-helix bundle (B) domain, which are similar to modules found in bacterial protein-histidine kinases (8) and the related pyruvate dehydrogenase kinase 2 (PDK2) of the mitochondrial pyruvate dehydrogenase complex (PDC) (9). The K domain is highly conserved between BCK and members of the GHL ATPase family (10), with the presence of characteristic N1, G1, F, and G2 boxes and an “ATP lid.” In BCK, the direct back-to-back interaction of two opposing K domains produces a dimeric structure in the crystal lattice. In contrast to the K domain, the functional significance of the extended B domain is unknown. Both ATPase (7) and phosphoryltransfer/kinase (11) activities of BCK are stimulated by lipoylated E2b. We have speculated that the E2b core binds to the B domain of BCK to promote the interaction between the B and K domains, resulting in the activation of BCK (7).

To approach this hypothesis, we have focused on the putative interaction of BCK with the N-terminal lipoic acid-bearing domain (LBD) of E2b, by analogy to the binding of the L2 domain of E2p to PDK (12). However, these studies have been hampered by the fact the lipoylated LBD (lip-LBD) alone (residues 1–84) cannot bind to BCK. Here we report that the previously overlooked C-terminal hinge region of LBD is essential for the interaction of lip-LBD with BCK. Constructs containing lip-LBD and various lengths of the C-terminal hinge region are able to bind to BCK to different degrees, as measured by a newly developed solubility-based assay and by isothermal titration calorimetry (ITC). The absence of relevant C-terminal hinge sequences may explain the previously observed weak binding of some PDK isoforms to the L2 domain of the PDC (13). More significantly, the inclusion of the C-terminal hinge region will facilitate investigations into the mechanism by which mitochondrial protein kinases, i.e. BCK and PDK isoforms, are regulated through interactions with their respective E2 cores of α-keto acid dehydrogenase complexes.

**EXPERIMENTAL PROCEDURES**

**Construction of Expression Plasmids for E2b Domains**—The di-domain (DD), which spanned residues 1–167 of human E2b, contained (5′→3′) the LBD, the complete C-terminal hinge region, and the subunit-binding domain (SBD). To construct the C-terminally His₆-tagged DD expression vector, a sense primer, 5′-CCATGGGACAGGTTGTTCAGT-3′, and an antisense primer, 5′-GCTCGAGTGGCATAATT-3′, were used.

---

* This work was supported by National Institutes of Health Grant DK-26758 and Welch Foundation Grant I-1286. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9038. Tel.: 214-648-2457; Fax: 214-648-8856; E-mail: David.Chuang@UTSouthwestern.edu

1 The abbreviations used are: BCKD, branched-chain α-keto acid dehydrogenase; BCK, BCKD kinase; DD, di-domain; lip-DD, lipoylated DD; E1b, branched-chain α-keto acid decarboxylase; E1p, pyruvate dehydrogenase; E2b, dihydrolipoyl transacaylase; E2p, dihydrolipoyl transacaylase; L2, inner lipoic domain; LBD, lipoic acid-bearing domain; lip-LBD, lipoylated LBD; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; SBD, subunit-binding domain; MBP, maltose-binding protein; ITC, isothermal titration calorimetry.
TCAGCTTTTGGTTG-3', were employed to amplify the DD using a human E2b cDNA as template (14). The amplified DD was digested with *NcoI* and *XhoI*, and the excised fragment was cloned into the Pet-28a vector from Novagen (Madison, WI). Similar strategies were used to construct vectors for the expression of LBD (residues 1–84), LBD2 (residues 1–99), LBD3 (residues 1–95), and LBD4 (residues 1–89).

**Expression and Lipoylation of DD and LBD Constructs**—C-terminally His6-tagged E2 domain constructs were transformed into BL-21 cells, and the expression at 30 °C was induced by 0.75 mM isopropyl-β-D-thiogalactopyranoside. Nickel-nitrotriacetic acid-purified DD and LBD proteins were lipoylated *in vitro* with the bacterial enzyme LplA as described previously (15).

Solubility-based Binding Assays for His6-tagged BCK—N-terminally His6-tagged BCK (40 μM, monomers) in 400 mM arginine was combined with lipoylated or unlipoylated DD or LBD constructs (70 μM). Each of the mixtures was dialyzed at 4 °C for 16 h against 50 mM Hepes buffer, pH 7.5, containing 50 mM KCl, 5 mM dithiothreitol, 1 mM MgCl2, and 5% glycerol. The dialyzed mixtures were clarified by centrifugation, and aliquots were analyzed by SDS-PAGE and Coomassie Blue staining.

**Binding Studies of Malto-hinge Protein** (MBP)-BCK with Lipoylated E2 Domain Constructs by Isothermal Titration Calorimetry—MBP-BCK and lip-DD or lip-LBD constructs were dialyzed exhaustively with three changes against the same reservoir of 50 mM potassium phosphate buffer, pH 7.5, 50 mM KCl, 20 mM β-mercaptoethanol, and 5% glycerol. To carry out ITC measurements, the reaction cell of a MicroCal (Northampton, MA) VP-ITC microcalorimeter contained 1.5 ml of 100 μM MBP-BCK was equilibrated at 20 °C. The stock of 500 μM lipoylated LBD or lip-LBD constructs in 10-μl increments was injected into the cell, and heat changes due to binding were recorded. Binding isotherms derived from the raw data were used to calculate the standard free energy of binding (ΔG°) according to the equation: ΔG° = RTlnKs, where R is the gas constant, T is absolute temperature, and Ks is the association constant. From binding isotherms, the number of binding sites (n) was obtained, and changes in enthalpy (ΔH°) and entropy (ΔS°) were calculated according to the equation: ΔG° = ΔH° - TΔS°. Curve-fitting and the derivation of thermodynamic parameters were facilitated using the MicroCal ORIGIN software package. The concentrations of MBP-BCK and E2 domain constructs were determined by A280, using calculated extinction coefficients (in mg ml⁻¹ cm⁻¹) of 1.09 (for MBP-BCK), 1.07 (LBD2), 1.14 (LBD3), 1.16 (LBD2), and 0.74 (DD).

**Other Methods**—The construction and expression of His6-tagged MBP and MBP-BCK by co-transformation with the pGroESL plasmid overproducing chaperonins GroEL and GroES were described previously (7, 11).

**RESULTS**

**Interactions of His6-tagged BCK with Lipoylated Di-domain**—A DD (residues 1–167) construct comprising the LBD, a hinge region, and the SBD was expressed and lipoylated *in vitro* and designated as lip-DD. The N-terminally His6-tagged BCK is soluble to the extent of 2–3 mg/ml only in the presence of 300 mM arginine (7). However, the requirement of high arginine concentration for solubility was circumvented when N-terminally His6-tagged BCK was allowed to bind to lip-DD. Based on this finding, a solubility-based binding assay was developed. Lip-DD was combined with the N-terminally His6-tagged BCK in the presence of 400 mM arginine. The mixture was dialyzed to remove arginine because it interferes with the binding of lipoylated or unlipoylated DD or LBD constructs (70 μM). Each of the mixtures was dialyzed at 4 °C for 16 h against 50 mM Hepes buffer, pH 7.5, containing 50 mM KCl, 5 mM dithiothreitol, 1 mM MgCl2, and 5% glycerol. The dialyzed mixtures were clarified by centrifugation, and aliquots were analyzed by SDS-PAGE and Coomassie Blue staining.

**Direct Binding Measurements by Isothermal Titration Calorimetry**—We utilized ITC as a direct method to study the interaction of lip-LBD2 with BCK. Measurements of heat changes by ITC require relatively large quantities of proteins especially when the binding energy is weak. For binding studies by ITC, rat BCK was expressed in *Escherichia coli* as a MBP fusion. MBP-BCK, unlike His6-tagged BCK, is highly soluble (~50 mg/ml) in the absence of arginine, and the presence of MBP has no effect on BCK activity and its interaction with lipoylated E2b (11). Fig. 3 (upper panel) shows the heat of titration with apo-LBD2 and lip-LBD2. The unchanged ITC prevent the precipitation of His6-tagged BCK upon the removal of arginine. The results indicate that the sequence of LBD alone is not capable of binding to BCK.

**The C-terminal Hinge Region of LBD Is Indispensable for the Interaction of LBD with His6-tagged BCK**—The ability of lip-DD, but not lip-LBD, to maintain the solubility of His6-tagged BCK in the absence of arginine raises the question of whether the C-terminal SBD or the hinge sequence between LBD and the SBD is needed for binding. Therefore, solubility-based binding studies with His6-tagged BCK were carried out further with lip-LBD constructs containing various lengths of the C-terminal hinge region (Fig. 2A). As shown in Fig. 2B, lip-LBD2 (residues 1–99) (lane 2) and lip-LBD3 (residues 1–95) (lane 4), both C-terminally His6-tagged, confer 100% solubility of His6-tagged BCK in the absence of arginine. His6-tagged BCK is absent from the supernatant with apo-LBD2 (lane 1) or apo-LBD3 (lane 3). The addition of lip-LBD4 (residues 1–89) (lane 6) results in lower levels of soluble His6-tagged BCK compared with lip-LBD2 and lip-LBD3. Identical amounts of His6-tagged BCK were used in the solubility-based binding assays with different constructs. Similarly, apo-LBD4 did not protect His6-tagged BCK from precipitation upon the removal of arginine (lane 5). No His6-tagged BCK was present in the supernatant in the presence of either apo-LBD (lane 7) or lip-LBD (lane 8), serving as negative controls. These results establish, for the first time, that the C-terminal hinge region of LBD is essential for the binding of lip-LBD to BCK. The hinge regions containing four negatively charged residues Glu167-Glu-Asp-Xaa-Xaa-Glu that are present in lip-LBD2 and lip-LBD3 are likely to confer efficient domain binding to BCK.

**Determining the Binding Affinity of His6-tagged BCK with Lipoylated LBD Constructs**—To determine the binding affinity of His6-tagged BCK with lipoylated LBD constructs, we utilized ITC (Fig. 4). The heat changes by ITC were affected by the length of the hinge region. For example, apo-LBD2 has a lower binding affinity compared with apo-LBD3. Similar results were obtained with apo-LBD4, apo-LBD5, and apo-LBD6. The binding affinity of His6-tagged BCK with apo-LBD6 was significantly lower than that with apo-LBD2 and apo-LBD3. The binding affinity of His6-tagged BCK with lip-LBD2, lip-LBD3, lip-LBD4, and lip-LBD6 was determined to be 2.0 × 10⁶ M⁻¹ for lip-LBD2, 5.0 × 10⁶ M⁻¹ for lip-LBD3, 5.0 × 10⁶ M⁻¹ for lip-LBD4, and 2.0 × 10⁶ M⁻¹ for lip-LBD6, respectively. The binding affinity of His6-tagged BCK with apo-LBD6 was determined to be 2.0 × 10⁶ M⁻¹ for apo-LBD2, 5.0 × 10⁶ M⁻¹ for apo-LBD3, 5.0 × 10⁶ M⁻¹ for apo-LBD4, and 2.0 × 10⁶ M⁻¹ for apo-LBD6, respectively. The binding affinity of His6-tagged BCK with apo-LBD6 was significantly lower than that with apo-LBD2 and apo-LBD3. The binding affinity of His6-tagged BCK with lip-LBD2, lip-LBD3, lip-LBD4, and lip-LBD6 was determined to be 2.0 × 10⁶ M⁻¹ for lip-LBD2, 5.0 × 10⁶ M⁻¹ for lip-LBD3, 5.0 × 10⁶ M⁻¹ for lip-LBD4, and 2.0 × 10⁶ M⁻¹ for lip-LBD6, respectively. The binding affinity of His6-tagged BCK with apo-LBD6 was determined to be 2.0 × 10⁶ M⁻¹ for apo-LBD2, 5.0 × 10⁶ M⁻¹ for apo-LBD3, 5.0 × 10⁶ M⁻¹ for apo-LBD4, and 2.0 × 10⁶ M⁻¹ for apo-LBD6, respectively.
isotherm with apo-LBD2 unequivocally demonstrates the absence of interactions with MBP-BCK (Fig. 3, lower panel). By comparison, lip-LBD2 shows a gradual rise in the isotherm, which is consistent with a weak binding to MBP-BCK. Because of the weak interaction, the titration is only ~80% saturated under the conditions used in these experiments. From the binding isotherm, a dissociation constant \( K_D \) of 8.06 \( \times 10^{-6} \) M, a binding energy \( \Delta G^0 \) of -6.84 kcal/mol, and a binding enthalpy \( \Delta H^0 \) of -3.68 kcal/mol were calculated (Fig. 3, lower panel). The data represent direct evidence for the interaction between lip-LBD2 containing the C-terminal hinge region and MBP-BCK.

**Thermodynamic Parameters for Interactions of LBD Constructs with MBP-BCK**—The ITC method was further employed to characterize the interactions of MBP-BCK with LBD constructs containing various lengths of the C-terminal linker region. Table I shows that the interactions of lip-LBD2 with MBP-BCK is the most exothermic among the four LBD constructs studied. The binding enthalpy \( \Delta H^0 \) of -3.68 kcal/mol is comparable with that of -3.70 kcal/mol measured with lip-DD. Significantly lower enthalpies were obtained with lip-LBD3 and lip-LBD4. Smaller differences in dissociation constants \( K_D \) were observed between lip-DD and lip-LBD2 through lip-LBD4. These thermodynamic parameters confirm the conclusions derived from the solubility-based binding assays that lip-LBD2 and lip-LBD3 interact with MBP-BCK to a degree similar to lip-DD.

**Discussion**

A prominent feature of the mitochondrial kinases comprising BCK and PDK isoforms is the up-regulation through interaction with their respective E2 cores of \( \alpha \)-keto acid dehydrogenase complexes (11, 17). The lipoyl-containing domains of the E2 cores function both as an anchor and a modulator for activities of the mitochondrial kinases. Studies with the lipoylated inner L2 domain have been complicated by the fact that the domain alone does not interact as efficiently as holo-E2p with the PDK isoforms, except for PDK3 (13, 18). In this communication, we show that lip-LBD alone does not bind to BCK; however, the inclusion of various lengths of the previously neglected C-terminal hinge region results in the interaction between the lip-LBD constructs and BCK. This information has facilitated the co-crystallization of BCK with lip-LBD constructs for the ongoing structural determination by x-ray diffraction (data not shown). The L2 domain of E2p used in binding studies contained either none (13) or a portion of the hinge region (12, 19). The omission of certain important elements in the C-terminal hinge region of the L2 domain may explain the weaker interaction of these lipoylated constructs with PDK isoforms than with holo-E2.

The essential determinants in the C-terminal hinge region of E2b LBD for the binding of lip-LBD to BCK are presently uncertain. However, the inclusion of negatively charged residues Glu-Glu-Asp-Xaa-Xaa-Glu in lip-LBD2 and lip-LBD3 constructs results in binding efficiency similar to lip-DD, based on dissociation constants and binding enthalpies measured by ITC. The lip-DD construct contains the entire hinge region connecting LBD and SBD and therefore mimics holo-E2 regarding binding to BCK. The results with the lip-LBD constructs strongly suggest that the negative charged residues in the C-terminal hinge region are candidate determinants for interactions with BCK. In the BCK structure (7), a groove formed between helices BH4 and BH3 of the B domain is lined with positively charged residues that are suited to interact with the negatively charged residues present in the C-terminal hinge region of LBD, as suggested for the PDK2 structure (9). On the other hand, the covalently attached lipoic acid is also essential for the binding of lip-LBD constructs to BCK. Due to the aliphatic nature of the lipoic acid moiety and the presence of hydrophobic residues in the hinge region, there may be additional weak hydrophobic interactions between lip-LBD constructs and BCK. The C-terminal hinge region of LBD is located 26 Å away from the lipoylated Lys-44 in the recently determined human LBD solution structure (20). Both structural elements are on the same side of the \( \beta \) barrel and repre-
sent two of the three most mobile regions in LBD (the remaining one being the large L1 loop), as indicated by the picosecond time scale of the spectral density function (1/H). These dynamic properties presumably facilitate the interactions of both the essential lipoic acid moiety and C-terminal hinge region with the B domain of BCK.

BCK does not form a stable complex with lip-DD or lip-LBD constructs to allow isolation by the conventional column chromatography or ultracentrifugation methods. We have therefore developed the solubility-based binding assay, which facilitates screening for the binding of domain constructs to BCK in a simple and straightforward fashion. The N-terminally His$_6$-tagged BCK is soluble only in the presence of high concentrations of arginine. The amphipathic character of arginine presumably protects exposed hydrophobic interacting surfaces of the folded protein (21). The crux of the binding assay is based on the discovery that a molar excess of lip-DD can increase the solubility of N-terminally His$_6$-tagged BCK from 2–3 mg/ml in the presence of 300 mM arginine to 20 mg/ml when arginine is replaced by lip-DD. However, arginine and lipoylated E2 domains are mutually exclusive in binding to His$_6$-tagged BCK as indicated by the phosphotransferase activity assays (data not shown). The slow gradual removal of arginine by dialysis from His$_6$-tagged BCK in the presence of lip-DD enables the latter to bind to BCK thereby maintaining its solubility. The molar excess of lip-DD or lip-LBD constructs in the binding assay ensures the optimal binding to His$_6$-tagged BCK upon the removal of arginine.

The ITC method offers “real time” measurements of thermodynamic parameters for protein-ligand and protein-protein binding reactions under defined solution conditions. ITC is ideal for studies of weak interactions of BCK with lip-DD and lip-LBD constructs by taking advantage of the high solubility of MBP-BCK. The dissociation constants ($K_D$) in the $10^{-6}$ range derived from ITC measurements for lip-DD and lip-LBD2, lip-LBD3, and lip-LBD4 are consistent with the weak interactions of BCK with these E2b domain constructs. These values are four orders of magnitude higher than those estimated for the tight binding of E1p or E3 to the DD of the PDC from *Bacillus stearothermophilus* (22). On the other hand, the binding enthalpy obtained by ITC measurements appears to be a more accurate parameter than the dissociation constant in differentiating binding affinity among lip-LBD constructs (Table I). The similar strongly exothermic binding enthalpies between lip-LBD2 and lip-DD indicate that the lip-LBD2 construct contains all the necessary determinants present in E2b for its interaction with BCK. Finally, for the fitting of the binding isotherm, a model based on one lip-LBD2 binding site per MBP-BCK monomer was used (Fig. 3). The best fit values generated a stoichiometry of $n \approx 0.5$, indicating that due to the weak interactions only one of the two sites in the MBP-BCK homodimer is occupied by lip-LBD2 under non-saturating conditions. This binding stoichiometry is consistent with that deduced from packing analysis of the BCK/lip-LBD2 crystals (data not shown).

Acknowledgments—We are indebted to Dr. Celestine Thomas for advice and assistance in ITC measurements and Dr. Mischa Machius for helpful discussions of x-ray crystallographic data on the BCK/lip-LBD2 crystals.

**REFERENCES**

1. Chuang, D. T., and Shih, V. E. (2001) in *The Metabolic and Molecular Basis of Inherited Disease* (Scrivcr, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Vogelstein, K. B., and Childs, R., eds) 8th Ed., pp. 1971–2006, McGraw-Hill Inc., New York.
2. Petti, F. H., Yeaman, S. J., and Reed, L. J. (1978) *Proc. Natl. Acad. Sci. U. S. A.* 75, 4881–4885.
3. Paxton, R., and Harris, R. A. (1982) *J. Biol. Chem.* 257, 14433–14439.
4. Damuni, Z., Merryfield, M. L., Humphreys, J. S., and Reed, L. J. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 4335–4338.
5. Harris, R. A., Hawes, J. W., Poger, K. M., Zhao, Y., Shimosura, Y., Sato, J., Jaskiewicz, J., and Hurley, T. D. (1997) *Adv. Enzyme Regul.* 37, 271–293.
6. Cook, K. G., Bradford, A. F., Yeaman, S. J., Atkin, A., Fearnley, I. M., and Walker, J. E. (1984) *Eur. J. Biochem.* 145, 587–591.
7. Machius, M., Chuang, J. L., Wynn, R. M., Tomchik, D. R., and Chuang, D. T. (2001) *Proc. Natl. Acad. Sci. U. S. A.* 98, 11218–11223.
8. Bilwes, A. M., Alex, L. A., Crane, B. R., and Simson, M. I. (1999) *Cell* 96, 131–141.
9. Steussyy, C. N., Popov, K. M., Bowker-Kinley, M. M., Sloan, R. B., Jr., Harris, R. A., and Hamilton, J. A. (2001) *J. Biol. Chem.* 276, 37443–37450.
10. Sosick, M. Robinson, V. L., and Goudreau, P. N. (2000) *Ann. Rev. Biochem.* 69, 183–215.
11. Davie, J. R., Wynn, R. M., Meng, M., Huang, Y. S., Aalund, G., Chuang, D. T., and Lai, K. S. (1995) *J. Biol. Chem.* 270, 19861–19867.
12. Liu, S., Baker, J. C., Andrews, P. C., and Roche, T. E. (1995) *Arch. Biochem. Biophys.* 316, 926–940.
13. Tuganova, A., Boulatnikov, I., and Popov, K. M. (2002) *Biochem. J.* 366, 361–366.
14. Chuang, J. L., Cox, R. P., and Chuang, D. T. (1997) *J. Clin. Invest.* 100, 736–744.
15. Chuang, J. L., Davie, J. R., Wynn, R. M., and Chuang, D. T. (2000) *Methods Enzymol.* 324, 192–200.
16. Radke, G. A., Ono, K., Ravindran, S., and Roche, T. E. (1993) *Biochem. Biophys. Res. Commun.* 190, 982–991.
17. Rice, T. E., Rahmatullah, M., Powers-Greenwood, S. L., Radke, G. A., Gopalakrishnan, S., and Chuang, C. L. (1989) *Ann. N. Y. Acad. Sci.* 573, 66–75.
18. Baker, J. C., Yan, X., Peng, T., Kasten, S., and Roche, T. E. (2000) *J. Biol. Chem.* 275, 15773–15781.
19. Mann, W. R., Dragland, C. J., Vinluan, C. C., Vedananda, T. R., Bell, P. A., and Perham, R. N. (1997) *Biochem. Biophys. Acta* 1480, 283–292.
20. Chang, C. P., Chou, H. T., Chuang, J. L., Chuang, D. T., and Huang, T. H. (2002) *J. Biol. Chem.* 277, 15865–15873.
21. Chuang, J. L., and Thomas, P. J. (1996) *J. Biol. Chem.* 271, 7261–7264.
22. Jung, H. I., Bowden, S. J., Cooper, A., and Perham, R. N. (2002) *Protein Sci.* 11, 1091–1100.