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Conserved Residues Are Functionally Distinct within Transketolases of Different Species†

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ABSTRACT: Most of the amino acid residues which interact with thiamine pyrophosphate are highly conserved among enzymes which use this cofactor. The possible roles of several such residues in cofactor binding, catalysis, and/or substrate binding were examined for human transketolase. Mutations in H110 resulted in dramatic reductions to 2% or less of the normal activity. No alterations were found in the $K_m$app's for the cofactor or for the donor and acceptor substrates. Alterations in Q428 resulted in a less severe loss of activity and also no changes in the $K_m$app's. On the basis of the results, H110, an invariant residue, is proposed to function as a base which abstracts a proton from the protonated 4′-iminopyrimidine ring. The deprotonated 4′-imino moiety is required for generation of the C2-thiazolium carbanion which attacks the donor substrate. Interestingly, the function in the human enzyme of this invariant histidine is distinct from its role in yeast transketolase in which it aids in binding donor substrate and in subsequent catalytic events. Q428 is suggested to play a supportive role by stabilizing and orientating a water molecule which mediates the interaction between the 4′-amino group and H110. In other TPP-utilizing enzymes, the equivalent residue of Q428 is a histidine and is thought to deprotonate the 4′-amino group.

Recent studies have more clearly elucidated the interactions between and catalytic mechanisms of thiamine pyrophosphate (TPP)[1] and several proteins which utilize this prosthetic group, including transketolase from *Saccharomyces cerevisiae* and humans. Crystallographic analysis of the yeast enzyme has provided a detailed picture of cofactor−amino acid interactions (Lindqvist et al., 1992; Nikkola et al., 1994) and has led to models of the catalytic mechanism and substrate binding (Schneider & Lindqvist, 1992). Human transketolase shows an overall identity of 24% with respect to the yeast enzyme (McCool et al., 1993) and 75% identity for the residues which interact with the TPP cofactor, many of which are invariant among all TPP-utilizing enzymes. Both human and yeast enzymes are active as a dimer, and both display hysteresis as reflected in a lag time in the progress curve which is highly dependent on TPP concentration (Booth & Nixon, 1993; Egan & Sable, 1981; Singleton et al., 1995). A slow dimerization step also contributes to the lag period for yeast transketolase but not for the human enzyme. This difference may be due to a much shorter connector in human transketolase between the two domains which provide most of the subunit−subunit interactions and which also participate in cofactor binding (McCool et al., 1993; Nikkola et al., 1994), the lack of complete conservation of the residues involved in subunit−subunit interactions (McCool et al., 1993), or the importance of posttranslational alterations in generating stable dimers for human transketolase (unpublisheed observations).

Figure 1 shows most of the predicted polar interactions between human transketolase and the thiazolium and 4-aminopyrimidine rings of the TPP moiety; a number of hydrophobic contacts are also involved but are not shown (Martin et al., 1995; Nikkola et al., 1994). For TPP-utilizing proteins, catalysis is initiated by formation of a carbanion at the C2 position of the thiazolium ring (De La Haba et al., 1955; Holzer et al., 1962; Kremer et al., 1979). For the yeast enzyme, Schneider and co-workers (Schneider & Lindqvist, 1992) have proposed that the invariant glutamate 418 (E366 of the human enzyme) protonates the pyrimidine ring, resulting in a resonance form with a positively charged 4′-imino group. The highly conserved histidine 481 (Q428 of the human enzyme) is proposed to abstract a proton from this group, resulting in the 4′-iminopyrimidine which in turn serves as a base to deprotonate the thiazole C2 atom. The essential role of glutamate 418 in this scheme has been established by site-directed mutagenesis (Wikner et al., 1994). Additionally, the invariant histidine 103 (H110 of the human enzyme) has been shown to be required for binding of the donor substrate and to facilitate catalysis presumably through maintenance of the orientation of the donor substrate and reaction intermediates (Wikner et al., 1995).

For the human enzyme, the glutamate 418 and histidine 103 equivalents are conserved. However, the equivalent residue of the yeast histidine 481 is glutamine 428 in human transketolase. This raises the question of which residue abstracts the proton in the human enzyme to generate the 4′-iminopyrimidine which serves as the base for generating the C2 carbanion. On the basis of yeast transketolase and

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[1] Abbreviations: IPTG, isopropyl thiogalactoside; NADH, nicotinamide adenine dinucleotide; PCR, polymerase chain reaction; R-5-P, ribose 5-phosphate; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TPP, thiamine pyrophosphate; Xu-5-P, xyluose 5-phosphate.

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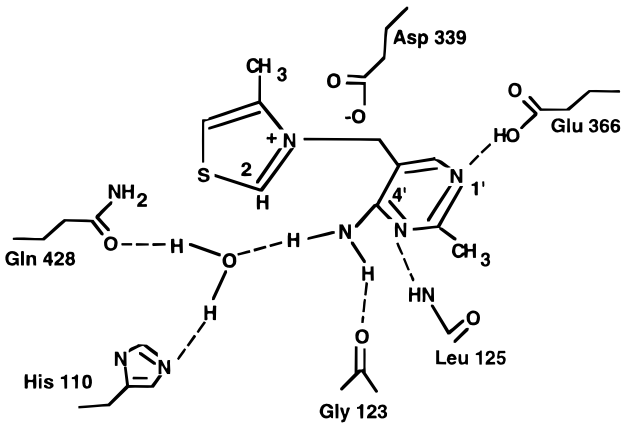

FIGURE 1: Proposed interactions between residues of human transketolase and the thiazolium ring and 4′-aminopyrimidine ring of thiamine pyrophosphate. Only the two ring systems of TPP are shown. Most of the polar interactions are shown and are predicted to occur on the basis of the crystal structure of yeast transketolase (Nikkola et al., 1994) and amino acid sequence comparisons between the yeast and human enzyme (Martin et al., 1995; McCool et al., 1993).

other TPP-utilizing proteins, glutamine 428 would have to be serving this function which seems unlikely. However, in the yeast enzyme, both histidines 103 and 481 interact with the 4′-amino group via water-bridged hydrogen bonding (shown in Figure 1 for the equivalent residues in the human enzyme), even though H481 is thought to remove the proton as H103 has been shown instead to be involved in donor substrate binding (Wikner et al., 1995). For human transketolase, it would thus seem that the histidine 103 equivalent (H110) may be responsible for the removal of the proton from the 4′-imino group and thus not involved in donor substrate binding and subsequent catalytic events as it is for the yeast enzyme.

In order to examine the possible roles of the equivalent human residues, we have generated and analyzed single and double mutants at Q428 and H110. Our results indicate that, in contrast to the situation in the yeast enzyme and presumably other TPP-utilizing enzymes, H110 is an essential residue in catalysis because of its role in generating the 4′-iminopyrimidine moiety, and it is not involved in donor substrate binding. Q428 is suggested to play a supportive role by stabilizing the orientation of a water molecule mediating the proton abstraction by H110, and it does not abstract the proton as its histidine equivalent is thought to do for the other enzymes.

MATERIALS AND METHODS

PCR-Based Site-Directed Mutagenesis. A construct (pHisTK-2) which allows for the production of wild type human transketolase possessing a histidine tag at the amino terminus was generated and has been described elsewhere (Wang et al., 1996). Mutations at the codon corresponding to H110 and Q428 were made using a two-step PCR procedure (Li & Shapiro, 1993). Briefly, the first step involved amplification with the mutagenic primer and another primer corresponding to a nearby region of the human transketolase cDNA on the opposite strand. The resulting mutant fragment served as the primer for a second amplification along with a primer corresponding to a nearby region on the opposite strand. The second amplified product was digested with two restriction enzymes, giving a fragment which possessed the desired mutation and different 5′ overhangs. The fragment was used to replace the unmutated fragment in pHisTK-2 which had been digested with the same two enzymes. Sequence analysis (Sanger et al., 1977) was carried out to confirm the presence of only the desired mutation and no other unintentional changes.

Purification of the Wild Type and Mutant Transketolases. Constructs were transformed into the DE3 strain of *Escherichia coli* (Fuerst et al., 1986). Cells were grown at 37 °C until an OD_{600} of 0.3−0.4 was reached; then 1 mM IPTG was added, and the culture was incubated for 3 h at 30 °C. The cells were pelleted and resuspended in 10 mL (per liter of culture) of Tris-HCl buffer (10 mM, pH 8.0). Lysozyme (30 μg) was added, and incubation was carried out at room temperature for 10 min. The sample was frozen in dry ice/ethanol and thawed at 37 °C, a procedure repeated three times. DNase I (100 μg/mL) was added, and the mixture was incubated at room temperature for 10 min. The sample was cleared by spinning for 10 min at 12000*g*. The supernatant was run on a nickel agarose column (1 mL of resin per liter of culture), and the column was washed with 20 mL of 50 mM imidazole, 100 mM NaCl, and 10 mM sodium phosphate (pH 8.0). Finally, His-transketolase protein was eluted with 0.5 M imidazole, 100 mM NaCl, and 10 mM sodium phosphate (pH 8.0) and dialyzed against 10 mM Tris-HCl (pH 8.0) overnight at 3 °C. Purity was assessed by SDS-PAGE (Laemmli, 1970).

Preparation of Apotransketolase. Saturated ammonium sulfate solution (3.4 mL) was added to 4.1 mL of purified mutant or wild type transketolase plus 2 mg of insulin (as carrier) in 10 mM Tris (pH 8.0) and 10 mM EDTA. After gentle mixing, the solution was allowed to stand at 0 °C for 20 min. The pH of the solution was then adjusted to 3.5 by the addition of H_2SO_4. After incubation at 0 °C for up to 30 min, the mixture was centrifuged at 30000*g* for 20 min. The pellet was resuspended in 20 mM Tris (pH 8.0) and used as a source of the apoenzyme. Successful removal of the cofactor was monitored by assaying for transketolase activity in the absence and presence of added magnesium and/or TPP.

Transketolase Assay. A typical assay consisted of sample protein, 100 mM Tris (pH 7.5), 10 mM ribose 5-phosphate, 2 mM xylulose 5-phosphate, 1.2 mM $MgCl_2$, 0.1 mM NADH, 1.2 u of α-glycerophosphate dehydrogenase/triosephosphate isomerase, and 1 mM TPP in a final volume of 0.6 mL (McCool et al., 1993). The nanomoles of NADH oxidized during the reaction were calculated from the change in absorbance (Smeets et al., 1971). The transketolase activity was expressed as nanomoles per minute per milligram or units per milligram. Apparent K_m values for TPP and V_{max} were determined using double-reciprocal plots (Lineweaver & Burke, 1934) of activities determined at various concentrations of TPP and derived from the steady-state region of each curve (Singleton et al., 1995).

RESULTS

The generation and characterization of His-tagged human transketolase was described previously (Wang et al., 1996). The specific activity and apparent K_m values for TPP, ribose 5-phosphate (R-5-P), and xylulose 5-phosphate (Xu-5-P) were essentially the same as those for highly purified transketolase from human cells.

Table 1: Properties of Transketolase Mutated at Residue 110

enzyme	% activity[a]	apparent K_m TPP[b]	apparent K_m Xu-5-P[c]	apparent K_m R-5-P[c]	V_{max}[d]
H110	100	0.73 ± 0.30	0.36 ± 0.24	0.39 ± 0.21	4800 ± 1000
H110A	1.9 ± 0.3	0.72 ± 0.01	0.43 ± 0.26	0.48 ± 0.12	49 ± 0.4
H110N	2.3 ± 0.7	0.55 ± 0.25	0.53 ± 0.13	ND	52 ± 3
H110Q	2.2 ± 0.3	0.89 ± 0.21	0.62 ± 0.27	ND	51 ± 4
H110E	0.3 ± 0.2	ND	ND	ND	ND

[a] Activity (nanomoles per minute per milligram) was determined for two independent isolations of each protein. The activities of the mutant proteins are expressed as a percentage of the activity of the unmutated protein (H110) which was set at 100% for each experiment. Values shown are the means ± mean deviation of the two independent determinations. [b] K_m expressed in micromolar. ND, not determined. [c] K_m expressed in millimolar. [d] V_{max} expressed in nanomoles per minute.

Table 2: Properties of Transketolase Mutated at Residue 428

enzyme	% activity[a]	apparent K_m TPP[b]	apparent K_m Xu-5-P[c]	apparent K_m R-5-P[c]	V_{max}[d]
Q428	100	0.73 ± 0.30	0.36 ± 0.24	0.39 ± 0.21	4800 ± 1000
Q428A	34.3 ± 1.7	0.96 ± 0.38	0.35	0.40	710 ± 10
Q428H	27.7 ± 1.7	0.22 ± 0.02	ND	ND	520 ± 80
Q428N	38.2 ± 0.3	0.94 ± 0.16	ND	ND	857 ± 230
Q428E	12.5 ± 2.4	2.3 ± 0.1	ND	ND	222 ± 111

[a] Activity (nanomoles per minute per milligram) was determined for two independent isolations of each protein. The activities of the mutant proteins are expressed as a percentage of the activity of the unmutated protein (Q428) which was set at 100% for each experiment. Values shown are the means ± mean deviation of the two independent determinations. [b] K_m expressed in micromolar. [c] K_m expressed in millimolar. ND, not determined. Only one determination was carried out for the mutant protein. [d] V_{max} expressed in nanomoles per minute.

Alterations at residue 110 resulted in a dramatic loss of activity (Table 1). Mutant enzymes in which the histidine was changed to alanine, asparagine, or glutamine gave only about 2% of the normal activity. A change to glutamic acid resulted in an enzyme with an activity which was at or just above the background and which was too low to allow examination of other parameters. The apparent K_m for the TPP cofactor and for the donor (Xu-5-P) and acceptor (R-5-P) substrates were unaltered in H110A, H110N, and H110Q. Thus, all of the reduction in activity was accounted for in a similar reduction in the maximum velocity.

Alterations at residue 428 resulted in a less dramatic but significant reduction of the activity (Table 2). Q428A, Q428H, and Q428N possessed about 30−40% of the activity of the wild type transketolase, while a change to glutamic acid was somewhat more deleterious. As with the 110 mutations, alterations at 428 gave little or no change in the apparent K_ms for the cofactor and substrates. The only effect seen was a slight (ca. 3-fold) increase in $K_m$app for TPP by the Q428E protein.

As expected from the above results, double mutants at both H110 and Q428 resulted in proteins with activities that were at or just above the background activity (Table 3). H110A/Q428A possessed the highest residual activity and was the only double mutant whose activity was consistently above the background.

Table 3: Relative Activities of Transketolase Mutated at Residues 110 and 428

enzyme	% activity[a]
H110/Q428	100
H110A/Q428A	1.5 ± 0.2
H110A/Q428H	0.3 ± 0.1
H110Q/Q428H	0.8 ± 0.1
H110E/Q428H	0.8 ± 0.1
H110N/Q428H	0.5 ± 0.2

[a] Activity (nanomoles per minute per milligram) was determined for two independent isolations of each protein. The activities of the mutant proteins are expressed as a percentage of the activity of the unmutated protein (H110/Q428) which was set at 100% for each experiment. Values shown are the means ± mean deviation of the two independent determinations.

DISCUSSION

Although no direct examination was carried out for potential structural perturbations resulting from the amino acid alterations that were made, several points suggest that such perturbations were negligible and probably not major factors in the observed reductions in activity. First, little or no change in $K_m$app was found for the TPP cofactor, the donor substrate, or the acceptor substrate. Thus, the affinities for all three appear to be unaltered, a situation unlikely if significant structural perturbations were present in the mutant proteins. Also, the equivalent residues in yeast transketolase not only project into the cofactor binding site but also line the bottom of a narrow "substrate" channel extending from the protein surface to the C2 atom of the thiazolium ring (Nikkola et al., 1994). These sites may thus present fewer potentially deleterious steric interactions when alterations are made. Finally, mutations of the equivalent residues in the yeast enzyme, including double mutants at both positions, have been made and result in no significant structural changes (Wikner et al., 1995).

Figure 1 shows many of the predicted polar interactions between human transketolase and the thiazolium and 4-aminopyrimidine rings of the TPP cofactor. Almost all of the key residues identified for yeast transketolase are conserved within the human enzyme. The importance of the E366 equivalent residue in yeast (E418) has been demonstrated (Wikner et al., 1994), and it is thought to donate a proton which leads to the charged 4′-iminopyrimidine resonance structure. The role of the H110 equivalent residue in yeast has also been examined (Wikner et al., 1995), and the

analysis demonstrated a role for this residue in binding the donor substrate and in catalysis, presumably through maintenance of the orientation of the donor substrate and reaction intermediates.

A significant nonconserved residue of human transketolase is Q428 which corresponds to H481 in the yeast enzyme, a residue that is a histidine in all other TPP-binding enzymes (Nikkola et al., 1994). This histidine has been proposed to be involved in removal of a proton to generate the 4′-iminopyrimidine thought to be required for carbanion generation at the C2 atom of the thiazolium ring (Schneider & Lindqvist, 1992; Wikner et al., 1994). Hence, the lack of an equivalent histidine in human transketolase raised the question of which residue of the human enzyme was responsible for proton abstraction from the charged 4′-imino moiety. From the proposed interactions of Figure 1, we reasoned that H110 might serve this function even though it serves a different role in the yeast enzyme and in presumably all other TPP-utilizing enzymes as it is an invariant residue (Wikner et al., 1995). Additionally, as both H110 and Q428 are proposed to interact with the 4′-amino group via a common water molecule, it was possible that alteration of Q428 to a histidine might generate a redundant mechanism for proton removal. These ideas were tested by mutating these two residues by themselves or in certain combinations of double mutations.

Alteration of H110 to any one of four other amino acids resulted in an enzyme with only 2% or less of the activity of the wild type transketolase. No change was found in the $K_m$app's for the TPP cofactor or the donor and acceptor substrates. These findings support the model that H110 in human transketolase serves as the base to deprotonate the charged 4′-iminopyrimidine, a model that is in contrast to the role of the equivalent histidine in the yeast enzyme and all other TPP-utilizing enzymes. Deprotonation would generate a 4′-imino moiety which is postulated to be responsible for carbanion formation at the C2 atom of the thiazolium ring (Schneider & Lindqvist, 1992). Evidence for the deprotonated N4′ position has been obtained in the crystal structure of pyruvate decarboxylase, another TPP-utilizing enzyme (Arjunan et al., 1996). The fact that residual activity was seen with the H110A, H110Q, and H110N mutants suggests that the water molecule mediating the interaction between the 4′-amino group and H110 was still bound and could facilitate proton removal, albeit at a much reduced efficiency. Alteration to glutamic acid resulted in an almost complete loss of activity, presumably due to interference with correct positioning of the water molecule.

An alternate mechanism for deprotonation of the C2 atom of the thiazolium ring does not involve the 4′-amino group (Harris & Washabaugh, 1995). The model is based on equilibrium isotope effect studies of the exchange of protons between enzyme function groups and the solvent for yeast pyruvate decarboxylase. Although their data provide no support for the role of the 4′-amino group as outlined above, their findings do not exclude such an involvement. The authors propose that the 4′-amino group is involved in stabilizing the initial enzyme-bound donor substrate intermediate. Although our finding of H110 mutations severely decreasing activity would be consistent with such a model, the fact that no change was found in the $K_m$app for the donor substrate would be inconsistent with such a proposal. Likewise, as mentioned, recent crystal studies on pyruvate decarboxylase from yeast demonstrate that the 4′-amino group is deprotonated and ready for acceptance of the hydrogen from the C2 atom (Arjunan et al., 1996).

The effects observed upon mutation of Q428 were interpreted to suggest that this residue stabilizes and aids in orienting the water molecule mediating the interaction between H110 and the 4′-amino group, in contrast to the postulated role of the equivalent residue in all other TPP-utilizing enzymes (Schneider & Lindqvist, 1992). Alteration to A or N resulted in a reduction of activity of about 60% with no alterations in $K_m$app's for TPP, the donor substrate, or the acceptor substrate. Q428E was again more deleterious, resulting in a loss of about 90% of the activity. A more severe "steric interference" with the proper positioning of the water molecule would also account for the slight increase in $K_m$app for TPP of the Q428E mutant protein. Q428H, which changes Q428 to that of the equivalent residue found in all other TPP-utilizing enzymes, was not able to support normal activity and resulted in a loss similar to that of the A and N mutants. Thus, changing the human enzyme at this site so that it is like other TPP-utilizing enzymes does not provide efficient catalysis, supporting the conclusion that the roles of the conserved residues are not identical between the human enzyme and other enzymes. When placed in a background of a mutated H110, the Q428H mutant further reduced the activity to at or just above the background. Q428 in the human enzyme thus plays an important supportive role for generation of the 4′-iminopyrimidine, but it cannot substitute for the H110 residue as the major base involved in proton removal. It thus differs in its function relative to the equivalent residue in other enzymes.

In conclusion, although many of the residues which are involved in cofactor and substrate binding and in catalysis are highly conserved among the various TPP-utilizing enzymes, our findings indicate that at least two residues, the H110 and Q428 equivalents, have quite different functions within the different enzymes. One of these functionally variable residues (H110) is invariant, and the other (Q428) is conserved as a histidine in all enzymes except for human transketolase. Although the physiological relevance of these functional differences is unknown, one implication is that they necessitate the involvement of different residues in binding the donor substrate and perhaps the acceptor substrate for human transketolase versus the yeast and other transketolases. The result may be transketolases possessing somewhat different repertoires of potential substrates as dictated by species differences in physiological substrates (Datta & Racker, 1961; Sprenger et al., 1995). Such a need for being able to utilize different substrates may explain why changes in the functional roles of these residues were initially brought about.

REFERENCES

Arjunan, P., Umland, T., Dyda, F., Swaminathan, S., Furey, W., Sax, M., Farrenkopf, B., Gao, Y., Zhang, D., & Jordan, F. (1996) *J. Mol. Biol. 256*, 590−600.

Booth, C. K., & Nixon, P. F. (1993) *Eur. J. Biochem. 218*, 261−265.

Datta, A. G., & Racker, E. (1961) *J. Biol. Chem. 236*, 617−623.

De La Haba, G., Leder, I. G., & Racker, E. (1955) *J. Biol. Chem. 214*, 409−426.

Egan, R. M., & Sable, H. Z. (1981) *J. Biol. Chem. 256*, 4877−4883.

Harris, T. K., & Washabaugh, M. W. (1995) *Biochemistry 34*, 14001−14011.
Holzer, R., Kattermann, R., & Busch, D. (1962) *Biochem. Biophys. Res. Commun. 7*, 167−172.
Kremer, A. j., Egan, R. M., & Sable, H. Z. (1979) *J. Biol. Chem. 255*, 2405−2410.
Laemmli, U. K. (1970) *Nature 227*, 680−685.
Li, X.-M., & Shapiro, L. J. (1993) *Nucleic Acids Res. 21*, 3745−3748.
Lindqvist, Y., Schneider, G., Ermler, U., & Sundstrom, M. (1992) *EMBO J. 11*, 2373−2379.
Lineweaver, H., & Burke, D. (1934) *J. Am. Chem. Soc. 56*, 658−666.
Martin, P. R., McCool, B. A., & Singleton, C. K. (1995) *Metab. Brain Dis. 10*, 189−194.
McCool, B. A., Plonk, S. G., Martin, P. R., & Singleton, C. K. (1993) *J. Biol. Chem. 268*, 1397−1404.
Nikkola, M., Lindqvist, Y., & Schneider, G. (1994) *J. Mol. Biol. 238*, 387−404.
Sanger, F., Nickle, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463−5467.
Schneider, G., & Lindqvist, Y. (1992) *Bioorg. Chem. 21*, 109−117.
Singleton, C. K., Pekovich, S. R., McCool, B. A., & Martin, P. R. (1995) *J. Nutr. 125*, 189−194.
Smeets, E. H. J., Muller, H., & Wael, J. D. (1971) *Clin. Chim. Acta 33*, 379−386.
Sprenger, G. A., Schorken, U., Sprenger, G., & Sahm, H. (1995) *Eur. J. Biochem. 230*, 525−532.
Wang, J.-L., Martin, P. R., & Singleton, C. K. (1996) *Biochem. J.* (submitted for publication).
Wikner, C., Meshalkina, L., Nilsson, U., Nikkola, M., Lindquist, Y., Sundstrom, M., & Schneider, G. (1994) *J. Biol. Chem. 169*, 32144−32150.
Wikner, C., Meshalkina, L., Nilsson, U., Backstrom, S., Lindqvist, Y., & Schneider, G. (1995) *Eur. J. Biochem. 233*, 750−755.

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