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Isolation and Properties of Human Transketolase

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Abstract—Recombinant human $(His)_6$-transketolase (hTK) was obtained in preparative amounts by heterologous expression of the gene encoding human transketolase in *Escherichia coli* cells. The enzyme, isolated in the form of a holoenzyme, was homogeneous by SDS-PAGE; a method for obtaining the apoenzyme was also developed. The amount of active transketolase in the isolated protein preparation was correlated with the content of thiamine diphosphate (ThDP) determined in the same preparation. Induced optical activity, facilitating studies of ThDP binding by the apoenzyme and measurement of the transketolase reaction at each stage, was detected by circular dichroism spectroscopy. A single-substrate reaction was characterized, catalyzed by hTK in the presence of the donor substrate and in the absence of the acceptor substrate. The values of the Michaelis constant were determined for ThDP and a pair of physiological substrates of the enzyme (xylulose 5-phosphate and ribose 5-phosphate).

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Transketolase (TK, EC 2.2.1.1), a thiamine diphosphate (ThDP)-dependent enzyme, was discovered in 1953. It has now been detected in almost every tissue studied, whether of animal or plant origin, as well as in microorganisms [1-3]. TK catalyzes one of the key reactions of the pentose phosphate pathway of carbohydrate transformation, i.e. cleavage of a keto sugar (donor substrate) at the C–C bond neighboring the keto group, followed by the transfer of the two-carbon fragment thus formed to an aldose (acceptor substrate). Human TK is a homodimer with two active centers, each of which binds noncovalently Mg^{2+} and ThDP [1, 4]. The monomeric unit comprises 623 amino acid residues (a.a.) and has a molecular weight of 67.9 kDa [1].

TK is characterized by broad substrate specificity [1, 3]. Its classical substrates are represented by donor/acceptor pairs of the pentose phosphate pathway of carbohydrate transformation. TKs isolated from different sources exhibit somewhat different substrate specificities. In mammals, the enzyme is more specific than its counterparts from baker's yeast or plants, using as donor substrates only phosphorylated ketoses (xylulose 5-phosphate (X-5-P) or sedoheptulose 7-phosphate) and phosphorylated aldoses (ribose 5-phosphate (R-5-P) or glyceraldehyde 3-phosphate (3-GAP)) and free glycolaldehyde (GA) as acceptor substrates [1, 3].

The enzyme from baker's yeast is the best studied TK species [1-3]. Baker's yeast TK was the first thiamine diphosphate-dependent enzyme subjected to X-ray crystal structure analysis (in 1992) [5, 6]. It can now be regarded as a model enzyme widely used for assessing methodological approaches to studying the properties of TKs isolated from any other source.

TK from human tissues (hTK) has not been studied adequately, the interest being largely related to its role in the development of neurodegenerative diseases [7]. Initially, hTK was isolated from human erythrocytes [8]. Hemolysates were purified on DEAE-Tris-acryl columns followed by adsorption to hydroxylapatite. This procedure made it possible to achieve 10,000-fold purification of the enzyme, and the preparations obtained had a specific activity of 6 U/mg (at 37°C). Others succeeded in obtaining 1.1 mg protein with specific activity of 2.38 U/mg from 800-900 ml blood [9].

In 1993, a system was proposed for heterologous expression of the gene encoding hTK in *E. coli* cells [10] with subsequent isolation of the polyhistidine-tagged

Abbreviations: DHEThDP, α,β-dihydroxyethylthiamine diphosphate; DTT, dithiothreitol; GA, glycolaldehyde; 3-GAP, glyceraldehyde 3-phosphate; GAPD, glyceraldehyde-3-phosphate dehydrogenase; hTK, transketolase from normal human tissues; R-5-P, ribose 5-phosphate; ThDP, thiamine diphosphate; TK, transketolase; TKTL1, transketolase from human tumor tissues, X-5-P, xylulose 5-phosphate.

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recombinant enzyme [11]. In addition to hydroxylapatite adsorption, the enzyme purification included ammonium sulfate precipitation and affinity chromatography using Ni-NTA-Sepharose. This approach made it possible to isolate from 10 liters of culture fluid 2.1 mg recombinant protein with specific activity of 13.5 U/mg (at 37°C). The low yield of the enzyme was attributed to possible aggregation of protein molecules, the presence of rare codons in cDNA of TK, and the difference in the content of tRNA between the human and *E. coli* cells.

No new data on the properties of hTK have been reported in recent years. Interest in hTK is related to its putative role in the development of tumor tissues. Tumor growth is known to be associated with an increase in the transketolase activity of the affected tissues. TK inhibitors cause a dramatic decrease in tumor growth rate. Thus, it was believed that TK operation is intimately involved in the formation and development of tumors [12]. A number of reports have been published that viewed TK inhibitors as prospective agents for chemotherapeutic treatment of tumors. However, the search for such inhibitors has not met with success. Proponents of this approach to tumor treatment assumed that hTK is encoded by a single gene, and, therefore, that the same TK form is expressed in normal and transformed tissues. Subsequent studies demonstrated that the enzyme of tumor cells is represented by a distinct TK form (TKTL1), which differs from its counterpart of normal human tissues by both the primary structure and amino acid composition. Amino acid sequences of hTK and TKTL1 are 77% homologous. The major characteristic distinguishing TKTL1 from hTK is the lack of 38 a.a. in the vicinity of the N-terminus that are present in the enzyme of normal tissues.

The presence of TKTL1 in tumor tissues has been used since 2005 as a marker for early detection of invasive growth [13]. Nevertheless, no reports of TKTL1 isolation and characterization have been published. Increased interest in the two TK forms expressed, respectively, in normal and malignant human tissues calls for a detailed study of the properties of the enzyme.

MATERIALS AND METHODS

Chemicals used in this work included NAD^+, glyceraldehyde-3-phosphate dehydrogenase (GAPD) from rabbit muscle, ThDP, β-hydroxypyruvate, Li salt, and R-5-P from Sigma (Germany), glycolaldehyde from Aurora (USA), dithiothreitol (DTT) and $CaCl_2$ from Fluka (Germany), agar, bacto tryptone, and arabinose from DIFCO (USA), and Ni-NTA-agarose from Invitrogen (Germany). X-5-P was synthesized as described previously [14]; the preparation obtained was 88% pure and did not contain aldehyde contaminants.

Measurement of hTK concentration. In the past, hTK concentration was determined by the method of Lowry (with BSA as a standard) or using an averaged value for the absorption coefficient, $A_{280}^{0.1\%} = 1.0$ $(mg/ml)^{-1}\cdot cm^{-1}$ [9]. The exact value of the absorption coefficient of hTK is presently unknown. The values of the absorption coefficient of hTK, calculated by us from its amino acid sequence using the ProtParam software [15], equaled 0.71 and 0.8 $(mg/ml)^{-1}\cdot cm^{-1}$, respectively, for the apoenzyme and the holoenzyme (apoenzyme complexed with two ThDP molecules).

Activity measurements. TK activity was measured using two methods. In the first approach, it was assessed by the rate of NAD^+ reduction, with GAPD as an ancillary enzyme and X-5-P/R-5-P as the substrates [16]. The reaction mixture had the following composition (final volume, 1 ml): 50 mM glycylglycine, 10 mM sodium arsenate, 0.37 mM NAD^+, 3 U GAPD, 3.2 mM DTT, 2.5 mM $MgCl_2$, 0.2 mM ThDP, 0.25 μg TK, 0.25 mM X-5-P, and 1.0 mM R-5-P (pH 7.6). The reaction was initiated by adding the substrates. Measurements were taken in 1-cm cuvettes at 340 nm, using an Aminco DW 2000 spectrophotometer (USA).

In the second approach, the activity was measured by the rate of oxidation of the α-carbanion intermediate in the presence of ferricyanide. The reaction is based on the ability of ferricyanide to oxidize the carbanion intermediate, α,β-dihydroxyethylthiamine diphosphate (DHEThDP), to glycolic acid [17] according to the following equation:

$$DHEThDP^- + 2Fe(CN)_6^{3-} + H_2O \xrightarrow{TK} ThDP +$$

$$+ CH_2OH{-}COOH + 2Fe(CN)_6^{4-} + 2H^+.$$

Measurements were taken in 1-cm cuvettes using the Aminco DW 2000 spectrophotometer operating in double-beam mode. The initial reaction rate remained constant for the first 30 sec. The decrease in the absorption, resulting from ferricyanide reduction, was recorded at 420 nm. The reaction mixture had the following composition (final volume, 2 ml): 50 mM glycylglycine, 2.5 mM $MgCl_2$, 0.1 mM ThDP, 1.25 mM $K_3[Fe(CN)_6]$, 0.25 mM X-5-P, and 25-60 μg TK (pH 7.6). The reaction was initiated by adding the enzyme; no addition was made to the control cuvette.

Circular dichroism spectra were recorded in 1-cm cuvettes at 20°C using a Jasco J810 or Mark V spectrometer (Jobin Ivon, France), upgraded with and operated by a PS1 computer (IBM, USA). Measurements were taken in 50 mM glycylglycine buffer (pH 7.6).

Preparation of apoenzyme. Saturated ammonium sulfate solution (pH 3.5) was added to the solution of hTK (0.5 mg/ml) in 10 mM glycylglycine buffer (pH 7.6) at the ratio 3 : 2, followed by incubation on ice and centrifugation (12,000*g*, 4°C) for 5 and 15 min, respectively. The supernatant was aspirated using a pipette, and the pellet dissolved in 50 mM glycylglycine buffer, pH 7.6.

Measurement of K_m for ThDP. Apo-hTK was preincubated (22°C) with variable concentrations of ThDP

(from 28 nM to 28 μM) in 50 mM glycylglycine buffer (pH 7.6) containing 2.5 mM $MgCl_2$ for 45-60 min. Following reconstitution of holo-hTK, other components required for measuring TK activity were introduced into the cuvette.

Measurement of ThDP content in holoenzyme preparation. ThDP was separated from holo-hTK using two approaches described below. In the first approach, 0.1 ml holo-hTK solution in 50 mM glycylglycine (pH 7.6) was boiled for 90 sec, cooled on ice, and centrifuged, after which the supernatant was collected and the pellet, discarded [2].

The second approach consisted of adding perchloric acid (to final concentration of 5%) to 0.1 ml holo-hTK solution in 50 mM glycylglycine (pH 7.6). The mixture was incubated on ice for 15 min and centrifuged, after which the supernatant was collected and pH adjusted to 6.5 using 1 M KOH. After 5 min, the mixture was centrifuged again and the supernatant collected.

The concentration of ThDP in the supernatants thus obtained was measured enzymatically by the method of Racker [2]. For this, the activity of baker's yeast TK was plotted against ThDP concentration. Each sample in this experiment had the following composition: 50 mM glycylglycine buffer (pH 7.6), 1 U baker's yeast apo-TK, 2.5 mM $CaCl_2$, and 0, 10, 20, 30, 40, or 50 μl of 1 μM ThDP solution (total volume, 100 μl). After 30 min, 10-μl aliquots were drawn and introduced into the system for activity determination using method 1 (described above).

To determine the unknown concentration of ThDP, the solution under study (10-30 μl) was incubated under the same conditions (without addition of external ThDP) for 30 min. Thereafter, a sample was drawn from the incubation mixture and introduced into the system for activity determination. The amount of ThDP in the sample under study was determined by the rate of TK reaction from the calibration plot.

RESULTS AND DISCUSSION

Obtaining transketolase. *Escherichia coli* strain BL21AI, transformed by the plasmid pDEST-17, was used for hTK isolation. The cells were grown in dYT medium (16 g/liter tryptone, 10 g/liter yeast extract, 5 g/liter NaCl, 100 mg/ml ampicillin). The suspension of the cells, stored frozen at −20°C, was thawed, and a 50-μl aliquot drawn and introduced into 5 ml of the medium. The medium containing the cells was incubated under continuous shaking for 18 h (37°C), followed by addition of 400 ml dYT medium (no induction) or dYT supplemented with 2 g/liter arabinose (induction). Thereafter, the cells were incubated (37°C) overnight under continuous shaking. All subsequent operations involving the enzyme isolation were performed in the cold.

Cells harvested by centrifugation (4000*g*, 4°C, 5 min) were resuspended in 5 ml lysis buffer (50 mM glycylglycine, 0.5 M KCl, pH 7.6), and the suspension was centrifuged once again (4000*g*, 4°C, 5 min). The pellet was resuspended in a twofold volume of the lysis buffer and sonicated until complete disruption of the cells; to avoid heating, sonication was performed in cycles of 20 sec at 90-sec intervals. Cell debris was pelleted by centrifugation (15,000*g*, 4°C, 15 min).

The extract thus obtained was loaded on a column packed with 0.8 ml Ni-NTA-agarose and pre-equilibrated with 50 mM glycylglycine buffer, pH 7.6. The column was washed in sequence with 0.5 ml lysis buffer (five times) and 0.5 ml wash buffer (three times); the wash buffer contained 50 mM NaH_2PO_4, 200 mM NaCl, and 20 mM imidazole (pH 7.0). The protein was eluted using imidazole buffer (50 mM NaH_2PO_4, 200 mM NaCl, and 300 mM imidazole, pH 7.0), salted out with ammonium sulfate (degree of saturation, 0.8), and pelleted by centrifugation. The pellet was then dissolved in 50 mM glycylglycine buffer (pH 7.6). The solution was stored at −18°C (with no activity loss throughout several months).

Effect of arabinose-induced expression on yield and specific activity of hTK. The enzyme isolated as described under "Materials and Methods" was homogeneous by SDS-PAGE (Fig. 1). It is clear from the figure that hTK exhibits higher mobility than its counterpart from baker's

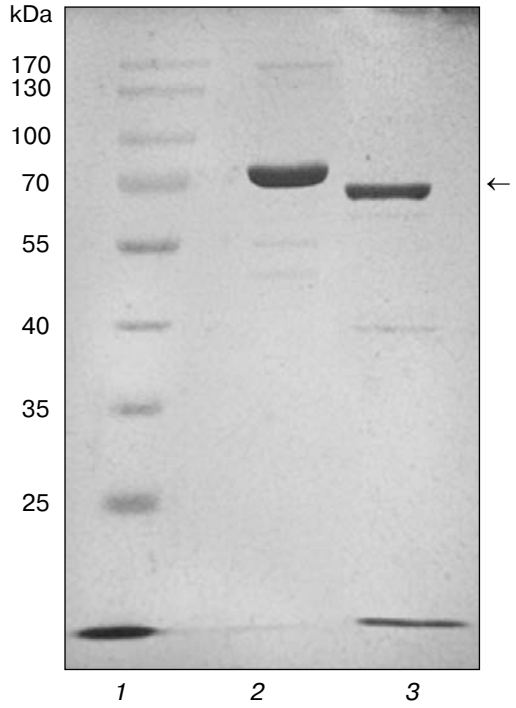


Fig. 1. Electrophoregram. Lanes: *1*) molecular weight markers; *2*) TK from baker's yeast, 1 μg; *3*) hTK, 0.75 μg (hTK is marked with an arrow).

Table 1. Effect of arabinose-induced expression on the yield and specific activity of hTK

Conditions	Yield of enzyme, mg	Specific activity, U/mg
Isolation 1		
without induction	3.30	2.20
with induction	0.36	0.07
Isolation 2		
without induction	6.3	3.64
with induction	0.69	0.17

Note: Protein was isolated from 120 ml culture fluid.

yeast (the respective molecular weights of a single subunit of each of these enzymes equal 67.9 and 74.2 kDa [1]). Here, as well as in all subsequent cases, we present reproducible results of typical experiments.

To assess the effect of arabinose-induced expression on hTK yield and specific activity, we isolated the enzyme from cultures grown both in the presence and in the absence of arabinose. As follows from Table 1, induction of the expression decreased the amount of hTK isolated and lowered its specific activity. The decrease in the yield of the enzyme associated with the induction was confirmed by electrophoretic data (lanes *1* and *2* in Fig. 2). It was assumed that the induction causes overexpression of the enzyme protein, most of which goes into inclusion bodies. This becomes evident from the comparison of lanes *3* and *4* in the electrophoretogram of Fig. 2. For this reason, all subsequent isolations were performed with no induction.

Maximum specific activity of hTK preparations was 3.64 U/mg at 20°C (yield, 6.5 mg per 120 ml culture fluid).

Determination of amount of active protein in hTK preparations. Differences in the specific activity between distinct preparations of the isolated protein suggest that each such preparation is a mixture of active molecules of holo-hTK (containing ThDP) and inactive molecules of the apoenzyme that are incapable of binding the coenzyme. Should this be the case, the specific activity would correlate with the amount of the coenzyme bound. Indeed, the preparations with specific activities in the range 1.8-2.0 U/mg contained 1 to 1.2 ThDP molecules per holo-hTK molecule, whereas preparations with specific activities of 1.0 U/mg contained less ThDP (0.7 molecule per holo-hTK molecule).

In this case, it is conceivable that the protein is a mixture of active dimers (each containing two bound ThDP molecules) and inactive dimers incapable of the coenzyme binding, or some dimers have two functional active centers, others have only one active center, and the remaining dimers are completely inactive.

Evidence in support of the second possibility was obtained when this matter was explored using TK from baker's yeast as a study object. Partial inactivation of this enzyme caused one of the two active centers to lose the capacity for binding the coenzyme (and, therefore, the catalytic activity). Conversely, the other active center fully retained its ability to bind ThDP and exhibit the catalytic activity [18].

Stability of hTK. The holoenzyme eluted from a Ni-NTA-agarose column using imidazole buffer (50 mM NaH_2PO_4, 200 mM NaCl, and 300 mM imidazole, pH 7.0) could be stored 4°C in the form of the eluate (protein concentration, ~1 mg/ml) for 24 h without any activity changes, whereas one cycle of freezing and thawing inactivated it completely. The holoenzyme dissolved in 50 mM glycylglycine buffer (pH 7.6) at concentrations in excess of 0.3 mg/ml was not affected by repeated freezing–thawing and retained activity when stored at −18°C (for several months) or 4°C (for several days). A dilute holo-hTK solution (0.1 mg/ml) was inactivated completely on freezing or following 24-h storage at 4°C. A 0.5-mg/ml solution of the apoenzyme in 50 mM glycylglycine buffer (pH 7.6) could be stored at 4°C without any loss of activity for several weeks; at −18°C, the stability of the apoenzyme was still higher, making possible its storage for longer periods.

Stability of ThDP–apo-hTK bond. Aliquots of holo-hTK solution (0.5 mg/ml) were placed into spectrophotometer cuvettes, diluted 100-fold by glycylglycine buffer

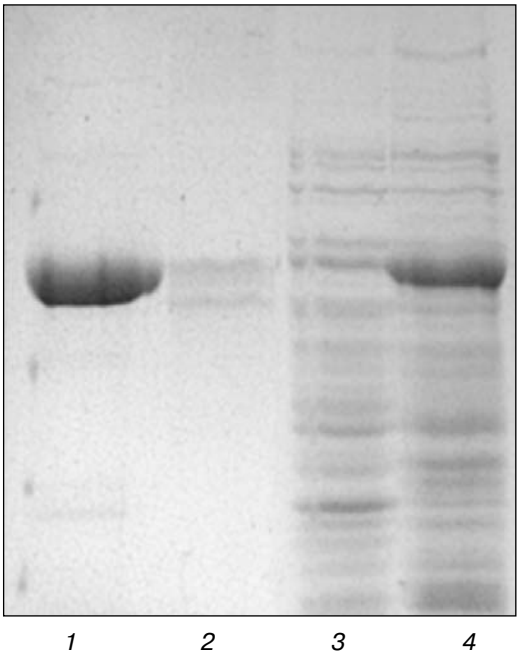


Fig. 2. Electrophoregram of hTK preparations isolated from cells grown without induction (*1*, *3*) or under the conditions of arabinose-induced expression (*2*, *4*). Lanes *1* and *2* correspond to the eluate (10 μl) from Ni-agarose column; lanes *3* and *4* correspond to aliquots (10 μl) of dissolved inclusion bodies.

Table 2. Influence of $MgCl_2$ and ThDP on hTK activity after treatment with acidic solution of ammonium sulfate

Cofactor added		Specific activity, %
$MgCl_2$, 2.5 mM	ThDP, 0.2 mM	
–	–	5
+	–	5
–	+	93
+	+	100

supplemented with 2.5 mM $MgCl_2$ (pH 7.6), and incubated at room temperature for 60 min. The enzymatic activity was measured at intervals using method 1 (see "Materials and Methods") without addition of Mg^{2+} and ThDP. Regardless of the incubation time, holo-hTK retained maximum activity (the rate of the reaction measured prior to diluting the enzyme was taken to represent maximum activity), i.e. ThDP did not dissociate from the active centers of holo-hTK under the conditions of the experiment. Our observation that the activity of the holoenzyme (without addition of external cofactors) did not change throughout storage was likewise indicative of strong binding of ThDP and the lack of its dissociation.

ThDP could be separated from the protein by treating holo-hTK with an acidic solution of ammonium sulfate. The apoenzyme thus obtained was rendered catalytically active by adding ThDP. Magnesium ions, alone or in combination with ThDP, did not affect the activity (Table 2).

Measurement of Michaelis constant for ThDP. The relatively slow reconstitution of holo-hTK, achieved by bringing together apo-hTK and the coenzyme, required preincubation of the components until completion of the process. This would take 45 to 60 min depending on the concentration of ThDP in the sample. Fitting of the data (dependence of the reaction rate on ThDP concentration) indicated that the value of K_m equaled 74 nM, which is in agreement with the literature (65 ± 14 and 85 ± 16 nM) [8].

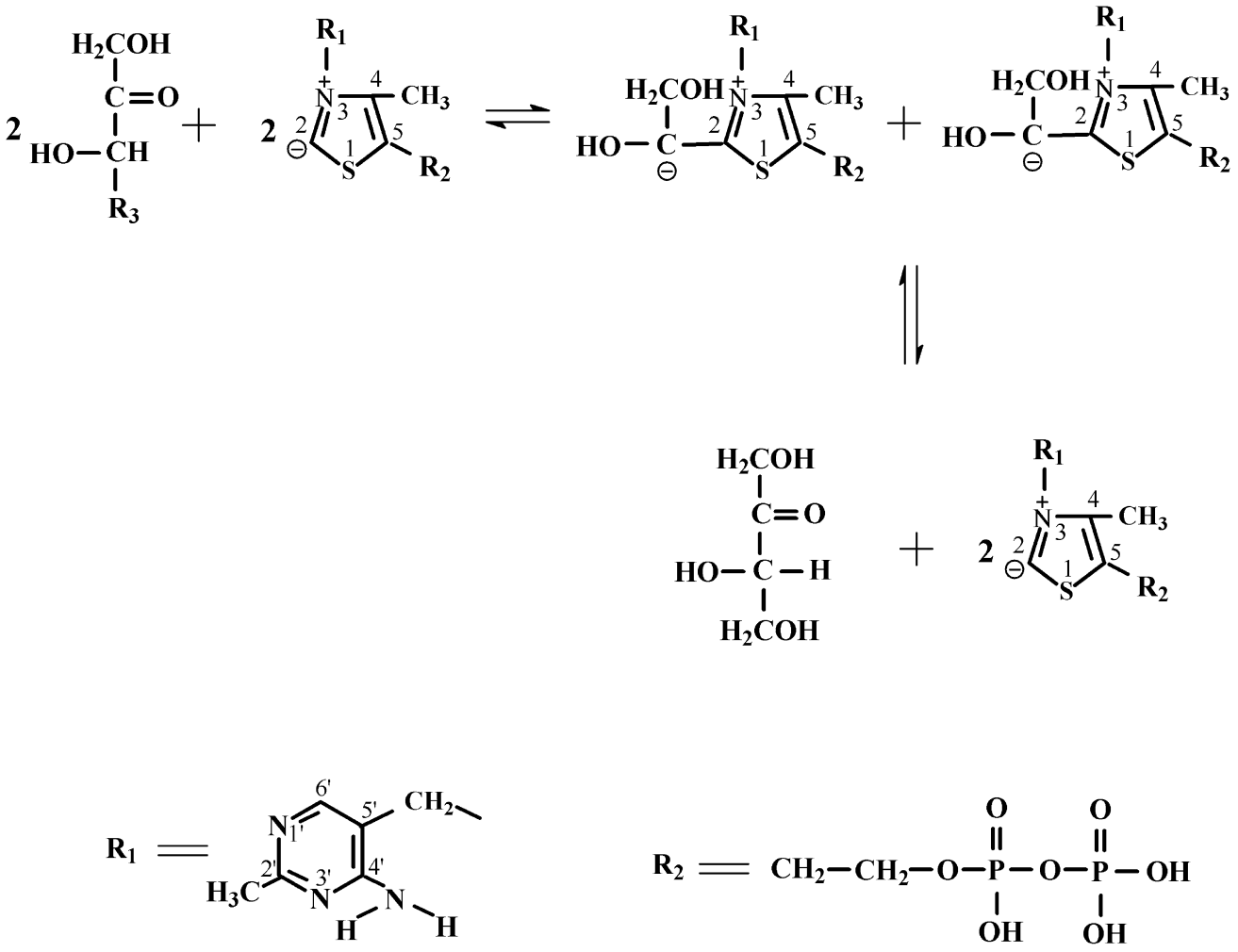
Measurement of Michaelis constant for X-5-P and R-5-P. To determine the affinity of hTK for the substrate, we studied the dependence of the reaction rate on X-5-P or R-5-P concentration. All other components of the system were taken in excess. The concentration of X-5-P was varied in the range 4.5-87.5 μM (the concentration of R-5-P in the sample was maintained at 1 mM). Similarly, the concentration of R-5-P was varied from 0.02 to 1 mM (the concentration of X-5-P was fixed at 175 μM). Fitting of the data indicated that K_m was equal to 11 and 63 μM for X-5-P and R-5-P, respectively.

The results did not match the published data (K_m = 270-360 μM for X-5-P; K_m = 390-510 μM for R-5-P) [11, 19]. The discrepancy could be due to differences in either the methods used for hTK isolation or the condition for measuring the kinetic parameters. As a rule, the affinity for one of the substrates was determined in the presence of unnecessarily high concentrations of the other substrate (e.g. 10 mM R-5-P) [11]. Consideration should also be given to the competition between R-5-P and X-5-P, documented for TK from baker's yeast (K_i = 3.8 mM) [20]. Perhaps such competition also takes place in the case of human TK.

Measurement of hTK activity in single-substrate reaction. Transketolase catalyzes reversible transfer of a glycolaldehyde residue from a ketose (donor substrate) to an aldose (acceptor substrate), acting as a typical transferase. With TK from baker's yeast, for example, it was nevertheless demonstrated that the enzyme is also capable of catalyzing single-substrate reactions such as ketose transformation in the absence of aldose [21, 22]. In the schematic depiction of a TK-catalyzed single-substrate reaction, glycolaldehyde formed as a result of donor substrate cleavage is further used by TK as an acceptor substrate for another glycolaldehyde residue, which results in the formation of erythrulose. When X-5-P serves as the sole substrate, the reaction products are represented by 3-GAP and erythrulose [21]. Experimental evidence was obtained that a single-substrate reaction involving 2 moles of X-5-P produces 1 mole of erythrulose. Although the single-substrate reaction is reversible, addition of ancillary enzymes eliminating 3-GAP from the reaction mixture moves the equilibrium towards the formation of 3-GAP and erythrulose [21]. The rate of the single-substrate reaction is relatively low, accounting for about 2% of that of its double-substrate counterpart [21].

It was of interest to check whether hTK is capable of catalyzing the transformation of X-5-P in the absence of the acceptor substrate. The activity of the enzyme, measured in a GAPD-coupled system with 1 mM X-5-P as a donor substrate and no acceptor substrate added, accounted for 6% of hTK activity in the double-substrate reaction. Thus, the relative rate of hTK-catalyzed single-substrate reaction was considerably higher than that of the reaction catalyzed by TK from baker's yeast (6 vs. 2%, respectively).

Optical characterization of hTK. ThDP binding by apo-TK from baker's yeast is associated with characteristic changes in its circular dichroism spectra: the amplitude of the positive absorption band at 270 nm is increased, and a negative (induced) band with a maximum at 320 nm appears [23, 24]. The emergence of this band characterizes the formation of the active center and of the catalytically active holoenzyme. A clear-cut linear relationship has been described between the amplitude of the induced absorption band and the amount of the reconstituted, catalytically active holoenzyme [25].



Schematic representation of single-substrate transketolase reaction

Optical characterization of TK from baker's yeast is used extensively in studies of this enzyme [25-28].

Optical properties of hTK have not been reported in the literature. Figure 3a shows circular dichroism spectra of apo-hTK and holo-hTK. As in the case of TK from baker's yeast, the spectrum of holo-hTK is characterized by a higher peak at 270 nm and the appearance of a negative band with an extremum in the vicinity of 320 nm. Thus, TK of normal human tissues (as well as TK from baker's yeast) exhibits induced optical activity, which can be used in studying this enzyme as well.

Circular dichroism spectroscopy and use of X-5-P as donor substrate make it possible to study transketolase reaction at each stage. Induced optical activity of TK from baker's yeast makes it possible to study the transketolase reaction at each of its two stages: (i) binding and cleavage of the donor substrate; and (ii) transfer of the two-carbon fragment to the acceptor substrate [29, 30]. Binding/cleavage of a ketose substrate drastically changes the circular dichroism spectrum, causing the negative extremum to disappear. Subsequent addition of an acceptor substrate restores the original appearance of the circular dichroism spectrum corresponding to that of the holoenzyme.

Similar to the case of TK from baker's yeast, addition of X-5-P (donor substrate) to holo-hTK eliminates the negative band and changes the shape of the positive peak (spectrum *3* in Fig. 3b). The changes in the spectrum are indicative of the formation of DHEThDP, an intermediate in the transketolase reaction. It should be noted that the spectrum of holo-hTK, taken in the presence of X-5-P, is clearly not identical to that of holo-TK from baker's yeast recorded under similar conditions. In the case of hTK, we observed, in addition to the disappearance of the negative band, its inversion. With holo-TK from baker's yeast, such spectra were recorded only in the presence of the irreversibly cleaved substrate (hydroxypyruvate) or in cases when the reaction of X-5-P cleavage is rendered irreversible by introduction into the system of the ancillary enzyme, GAPD [22].

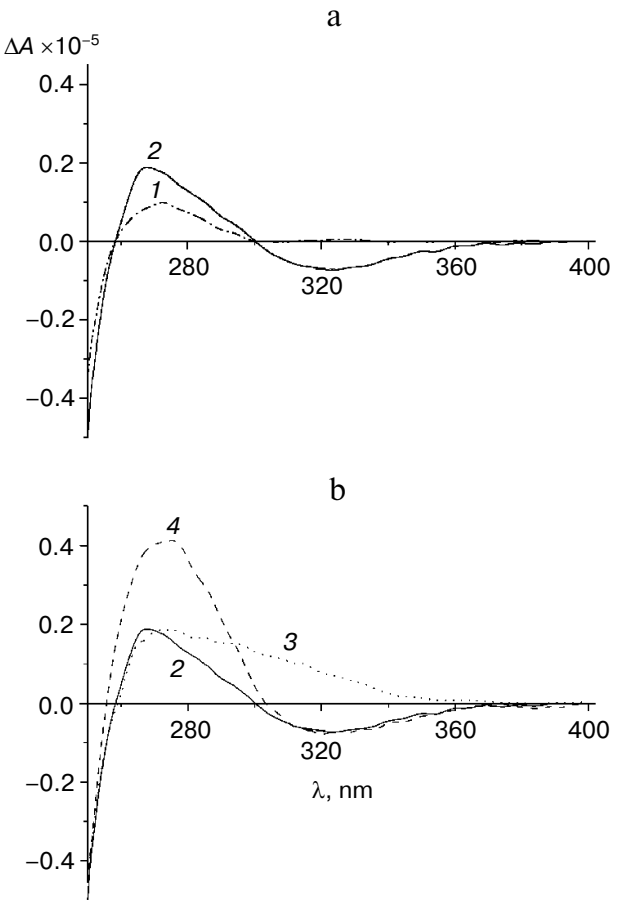


Fig. 3. Circular dichroism spectra of recombinant hTK: *1*) apoenzyme; *2*) holoenzyme; *3*) holoenzyme + X-5-P; *4*) holoenzyme + X-5-P + GA.

As observed previously with TK from baker's yeast, subsequent addition of GA (acceptor substrate) to the mixture holo-hTK + X-5-P restored the negative band characteristic of the holoenzyme; moreover, a new absorption band became apparent with a maximum at 275 nm characteristic of the reaction product, erythrulose (spectrum *4* in Fig. 3b). Therefore, the optical properties of hTK opens up possibilities for recording, in addition to ThDP binding by the apoenzyme, both stages of the transketolase reaction: binding and cleavage of the donor substrate (ketose) and transfer of the two-carbon fragment thus formed to the acceptor substrate (aldose). This latter possibility can be used in studies of the substrate specificity of the enzyme and in the search of its substrate-based inhibitors.

In conclusion, hTK differs from baker's yeast TK in several respects: (i) the affinities of the human enzyme for ThDP and both substrates are higher; (ii) the single-substrate reaction involving only the donor substrate (X-5-P) has a higher rate when catalyzed by hTK as compared to the yeast enzyme; (iii) effects of donor substrate on the circular dichroic spectrum are more pronounced in the case of hTK. Although the latter observation can be accounted for by the higher affinity of the enzyme for the substrate, the mechanisms underlying the difference remain to be elucidated.

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