High Control Coefficient of Transketolase in the Nonoxidative Pentose Phosphate Pathway of Human Erythrocytes: NMR, Antibody, and Computer Simulation Studies†

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ABSTRACT: The degree of control exerted by transketolase over metabolite flux in the nonoxidative pentose phosphate pathway in human erythrocytes was investigated using transketolase antiserum to modulate the activity of that enzyme. ^{31}P NMR enabled the simultaneous measurement of the levels of pentose phosphate pathway metabolites following incubation of hemolysates with ribose 5-phosphate. The variations in metabolic flux which occurred as the transketolase activity of hemolysate samples was altered indicated that a high degree of control was exerted by transketolase. Investigations using transaldolase-depleted hemolysates showed that transaldolase exhibits a lesser degree of control over pathway flux. Experimental data were compared with simulations generated by a computer model encompassing the reactions of the classical nonoxidative pentose phosphate pathway. The sensitivity coefficients (also called "control strengths" or "flux-control coefficients") calculated from the computer simulations were 0.74 and 0.03 for transketolase and transaldolase, respectively.

Two major metabolic pathways for glucose utilization operate in human erythrocytes: glycolysis and the pentose phosphate pathway or hexose monophosphate shunt. Metabolism through the pentose phosphate pathway provides the cell with ribose 5-phosphate for the biosynthesis of nucleotides; the formation of NADPH[1] in the oxidative branch of the pentose phosphate pathway affords the cell protection against oxidative damage.

A metabolic pathway may be characterized by such properties as flux and the steady-state concentrations of the metabolites. These, in turn, are the result of the interactions of all component enzymes with the substrates and intermediates which are being transformed. When the activity of one of the enzymes of a pathway is altered, the pathway flux will change and the metabolites will assume new steady-state values. The sensitivity coefficient or flux-control coefficient (Kacser & Burns, 1973; Heinrich et al., 1977) of an enzyme is a measure of the effect a small change in the activity of that enzyme will have on flux through the metabolic pathway. An enzyme with a control coefficient of 1, in which changing the enzyme activity results in a proportionate change in pathway flux, may therefore be termed "controlling"; an enzyme with a control coefficient of 0.01 exhibits a very low degree of control. A variety of techniques for identifying the enzyme of the nonoxidative pentose phosphate pathway in which control principally resides have been employed [for a review and discussion, see Wood (1985)]. These have included enzyme activity measurements, studies of the deviation of metabolite concentrations from equilibrium ratios, the construction of "cross-over" plots, and the identification of enzymes under hormonal or allosteric control or which respond to changes in the environment. Elevated transaldolase levels in *Saccharomyces cerevisiae* were used to demonstrate that this enzyme is probably not a rate-limiting step during xylose assimilation (Senac & Hahn-Hägerdal, 1991). In the study described in this paper, the response of metabolite levels and fluxes to modulations in transketolase and transaldolase activities were measured in order to evaluate the degree of control exerted by these enzymes in the pentose phosphate pathway of human erythrocytes. While transketolase has been generally ascribed a rate-limiting role in most tissues [e.g., in various rat tissues (Novello & McLean, 1968)], its control is not well-established. D-*glycero*-D-*ido*-Octulose 1,8-bisphosphate:D-*altro*-heptulose 7-phosphotransferase, an enzyme of the more recently proposed L-type formulation (Williams & Clark, 1971; Williams et al., 1978a,b) of the pathway, has also been postulated to be rate-determining (Williams, 1980; Arora et al., 1985; Butler et al., 1990).

Transketolase (EC 2.2.1.1) catalyzes the reversible transfer of a ketol moiety to a suitable aldehydic acceptor and displays a broad substrate specificity (Williams et al., 1987). It is proposed that it plays a role in both the classical and the L-type pathways. There are two primary transketolase reactions in the classical pathway and two in the L-type pathway with one proposed reaction in common.

In our investigation, ^{31}P nuclear magnetic resonance was used to measure the flux of metabolites through the nonoxidative pentose phosphate pathway in enzyme-depleted hemolysates. NMR has the advantages of enabling metabolite levels to be monitored continuously and noninvasively. The isotopic abundance of the ^{31}P nucleus, its ubiquity in biological tissues, and the simplifying aspect of there being a small number of atoms in each molecule renders it particularly suitable for biological studies [e.g., Gadian and Radda (1981)].

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[1] Abbreviations: DHAP, dihydroxyacetone phosphate; DPG, 2,3-diphosphoglycerate; E4P, erythrose 4-phosphate; F16P2, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; G3P, glyceraldehyde 3-phosphate; Glc6P, glucose 6-phosphate; h, hydrate form; k, ketone form; NADPH, β-nicotinamide adenine dinucleotide phosphate (reduced form); P_i, inorganic phosphate; Rib5P, ribose 5-phosphate; Ru5P, ribulose 5-phosphate; Sed17P2, sedoheptulose 1,7-bisphosphate; Sed7P, sedoheptulose 7-phosphate; Xu5P, xylulose 5-phosphate.

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The efficacy of computer modeling for investigating the effects of changes in levels of enzymes or substrates on the "whole" biochemical system has been demonstrated with the construction of a number of models describing red blood cell metabolism (Rapoport et al., 1977; Schuster et al., 1988; McIntyre et al., 1989; Joshi & Palsson, 1989, 1990; Kuchel et al., 1990). In this study, computer simulations of the nonoxidative pentose phosphate pathway, in which transketolase and transaldolase levels were modulated, were compared with experimentally derived NMR time-course results.

MATERIALS AND METHODS

Preparation of Hemolysates. Leucocyte-free human erythrocytes were obtained from freshly drawn venous blood (HAB), which was diluted 2:1 with ice-cold saline and filtered using a Sepacell R-500 leucocyte removal filter (Asahi Medical Co. Ltd., Tokyo, Japan). The resultant cell suspension was washed three times in isotonic saline, the last wash using saline constituted with 20% (v/v) ^2H_2O. The supernatant was removed by aspiration. The cell suspension was gently gassed with CO for ~10 min after the first wash. Hemolysates were prepared by sonication of the cell suspension (3 × 5 s, 40-W output, Branson sonifier model B-12, Danbury, CT). The hematocrit of the cell suspension before hemolysis was typically 0.85. Hemolysates were stored frozen at −20 °C. Prior to use, hemolysates were incubated at 37 °C for 40 min in order to deplete the lysate of NAD and NADP through the action of NAD(P)-glycohydrolase (Kuchel & Chapman, 1985). This results in the isolation of the reactions of the nonoxidative pentose phosphate pathway by preventing substrate recycling through the oxidative part of the pentose phosphate pathway or removal through glycolysis.

Enzyme Assays. Transketolase activity was determined by a coupled spectrophotometric assay [Horecker et al. (1953) with the modifications for hemolysate samples described by Smeets et al. (1971)]. To a 1-cm light-path cuvette was added 2.7 mL of ribose 5-phosphate, disodium salt (0.015 M), 0.1 mL of thiamine pyrophosphate chloride (0.01 M), 55 μL of dilute hemolysate sample, 50 μL of NADH (0.01 M), and a mixture of triosephosphate isomerase (EC 5.3.1.1) and glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) [10 mg/mL crystalline suspension in $(NH_4)_2SO_4$ (3.2 M) from Boehringer Mannheim, Sydney, NSW]. Reagents were freshly dissolved in 0.1 M Tris buffer, pH 7.6. The decrease in absorbance of NADH at 340 nm was recorded with a CARY 3 UV/vis spectrophotometer (Varian) beginning 15 min after the mixing of assay constituents. The temperature was maintained at 37 °C, and the fall in absorbance was monitored for 15 min. Transaldolase activity was determined by the measurement of glyceraldehyde 3-phosphate production with fructose 6-phosphate and erythrose 4-phosphate as substrates (Bergmeyer et al., 1974). The linear fall in absorbance at 340 nm was followed for 10 min beginning 5 min after mixing sample and reagents. Background absorbance changes were determined for all enzyme assays.

Preparation of Antitransketolase Serum. Human erythrocyte transketolase was purified as described previously (Abedinia et al., 1992) to yield a preparation homogeneous on electrophoresis in a polyacrylamide gel containing sodium dodecyl sulfate (Laemmli, 1970) and by successful sequencing of derived peptides up to 16 residues. Its specific activity was 12 units/mg. On three occasions 4 weeks apart, 50 mg of this preparation was injected into the thigh muscles of a rabbit after emulsification in Freund's adjuvant (complete adjuvant on the first occasion, incomplete on the subsequent occasions).

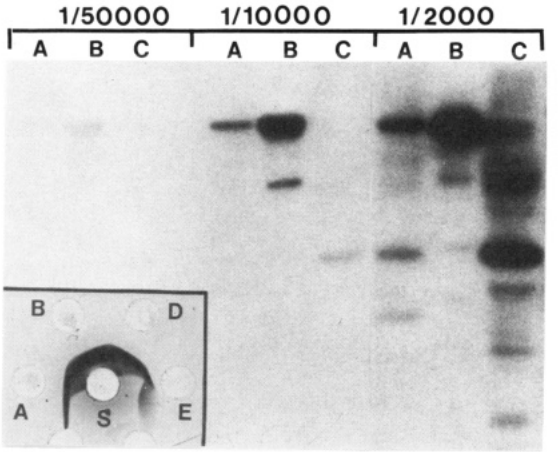

FIGURE 1: Reaction of serum with antigens. (Inset) Ouchterlony plate stained with Coomassie blue (0.05% w/v). S, undiluted serum; A, crude hemolysate; B, undiluted transketolase preparation; D, transketolase preparation diluted 5-fold; E, transketolase preparation diluted 10-fold. (Outer) Serum diluted 50 000-, 10 000-, and 2000-fold was reacted with electrophoretically separated, denatured, undiluted, Western-blotted antigens, and complexed rabbit immunoglobulins were detected by enhanced chemiluminescence. (A and B) As above; (C) crude extract of *E. coli*. For details, see Materials and Methods.

Two weeks after the last injection, the rabbit was exsanguinated under anesthesia and the serum separated from the clotted blood. The serum was used without further purification.

The titer and specificity of the undiluted antiserum were tested by diffusion in an Ouchterlony plate against undiluted and diluted native antigens, with Coomassie blue staining of the antibody–antigen complexes (Figure 1). Diluted sera were tested by reaction with denatured undiluted antigens on an Immobilon-P PVDF membrane (Millipore Corporation, Bedford, MA). The antigens had been separated by electrophoresis in a polyacrylamide gel containing sodium dodecyl sulfate (Laemmli, 1970) and were electrophoretically transferred to the membrane. Antigen–antibody complexes on the washed membrane were detected by use of the Enhanced Chemiluminescence (ECL) Kit (Amersham International, Amersham, U.K.) for the detection of membrane-bound rabbit primary antibodies, with signal capture by X-ray film (Kodak). Tested antigens were a crude human erythrocyte lysate, a purified human erythrocyte transketolase preparation of specific activity 12 units/mg, equal in purity to the antigen injected into the rabbit, and a crude extract of *Escherichia coli*.

Enzyme Inactivation in Hemolysates. Transketolase inactivation was effected by incubation of hemolysate (300 μL) and antiserum (various amounts, 0–880 μL) in Tris buffer (total volume = 1.8 mL) at 37 °C for 30 min. The Tris buffer had the following composition: Trizma-HCl (Sigma Chemical Co., St. Louis, MO), 0.1 M for the time course in which the transketolase level was 56%, 0.2 M for the other experiments; $MgCl_2$, 2 mM; EDTA, 0.5 mM; DL-dithiothreitol (Sigma), 1 mM; ^2H_2O, 10% (v/v); pH 7.4, adjusted by addition of KOH at 37 °C. (A control experiment in which antiserum was replaced with isotonic saline was conducted in conjunction with each antiserum experiment.) Samples were then centrifuged in an Eppendorf centrifuge for 5 min, and the supernatant was withdrawn for subsequent ^{31}P NMR time-course experiments and spectrophotometric transketolase activity determinations.

Inactivation of transaldolase was achieved by heating hemolysates at 55 °C for 5 min (Takeuchi et al., 1984). Modulation of transaldolase activity was achieved by mixing

quantities of normal and transaldolase-inactivated hemolysates in varying proportions.

^{31}P NMR Spectra. To an 8-mm diameter NMR tube containing ribose 5-phosphate (20 mM) was added Tris buffer, methyl phosphonate, triethyl phosphate, and hemolysate/antiserum (or NaCl) mixture (total volume, 1.8 mL; final concentration of hemolysate equivalent to that which would be obtained from a cell suspension of hematocrit 0.10). This NMR tube was held in a 10-mm outer diameter NMR tube with ^2H_2O in the surrounding annulus. Acquisition of ^{31}P NMR spectra was begun approximately 3 min after sample additions, and time courses were followed for 480 min. Triethyl phosphate served as a chemical shift reference and methyl phosphonate enabled continuous measurement of pH to be achieved (Slonczewski et al., 1981; Labotka & Kleps, 1983; Stewart et al., 1986).

NMR spectra were obtained using a Bruker AMX-600 spectrometer at 242.94 MHz for ^{31}P observation. Samples were spun at ~10 Hz to average magnetic field inhomogeneities. Broad-band proton irradiation (WALTZ-16; Shaka et al., 1983) was applied in all spectra. Sample temperatures were kept at 37 °C by setting the probe temperature below this value to compensate for the heating effect of the broad-band irradiation. The sample temperature was measured by acquiring a single-pulse ^1H spectrum of ethylene glycol contained in a capillary, immediately after switching off the broad-band decoupling, according to the method of Bubb et al. (1988). Spectra were acquired with a pulse angle of 90° and a repetition time of 20 s (>5 × T_1 for all sugar phosphates); 80 FID were used for each spectrum, and the spectral width was 7350 Hz. Spectra were processed without line broadening and were zero-filled once. Integration of peaks was performed using Bruker integration software (version 901001.5), and metabolite concentrations were derived using the principle of conservation of mass.

With the NMR time courses in which transaldolase activity was varied, hemolysate (1.3 mL) was added to ribose 5-phosphate (20 mM) in Tris buffer (total sample volume, 3.0 mL; final hematocrit equivalent, 0.36). Triethyl phosphate and methyl phosphonate again served as chemical shift and pH references. The total sample volume (3 mL) was contained in a 10-mm diameter NMR tube. Acquisition of ^{31}P NMR spectra began approximately 3 min after sample mixing, and time courses were followed for 128 min.

NMR parameters and conditions were as described above except 48 FID were acquired for each spectrum.

Computer Simulations. Computer simulations were carried out using the SCoP computer package (National Biomedical Simulation Resource, Duke University Medical Center, Durham, NC; NIH Grant RRO1693). The relationships between the steady-state kinetic parameters and unitary rate constants were determined, following derivation of the rate equations for the enzyme reactions according to their published mechanisms (McIntyre et al., 1989). The values of the unitary rate constants were changed iteratively by hand until a set was obtained which was consistent with the published steady-state values and, when incorporated into the computer model, simulated metabolic flux profiles which were in accordance with those obtained experimentally. The model has also been simplified to include only the reactions of the nonoxidative pentose phosphate pathway (Figure 2) and contains 46 unitary rate constants and seven different enzymes. The simulations were carried out on a Silicon Graphics 4D-20 IRIS computer.

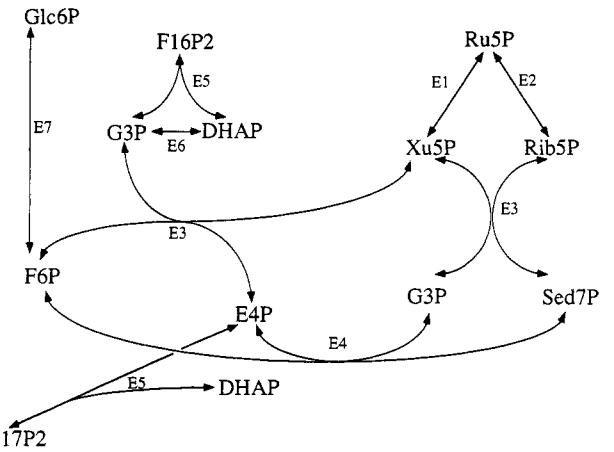

FIGURE 2: Nonoxidative pentose phosphate pathway reaction scheme used for computer simulations. The reactions are catalyzed by E1, ribulose-5-phosphate epimerase; E2, ribose-5-phosphate isomerase; E3, transketolase; E4, transaldolase; E5, aldolase; E6, triosephosphate isomerase; E7, glucose-6-phosphate isomerase. The mechanisms by which these enzymes are assumed to operate and the definitions of the unitary rate constants and equation numbers are the same as those of McIntyre et al. (1989). The unitary rate constants used in the computer model were (s^{-1} and M^{-1} s^{-1} for first- and second-order rate constants, respectively) E1, $k_1 = 3.91 \times 10^6$; $k_2 = 4.38 \times 10^2$; $k_3 = 3.05 \times 10^2$; $k_4 = 1.49 \times 10^6$; E2, $k_1 = 2.16 \times 10^4$; $k_2 = 1.42 \times 10^1$; $k_3 = 3.33 \times 10^1$; $k_4 = 6.09 \times 10^4$; E3 (eq 1), $k_1 = 2.16 \times 10^5$; $k_2 = 5.5 \times 10^1$; $k_3 = 7.7 \times 10^1$; $k_4 = 9.0 \times 10^6$; $k_5 = 2.632 \times 10^5$; $k_6 = 2.53 \times 10^2$; $k_7 = 7.7 \times 10^1$; $k_8 = 1.3 \times 10^3$; (eq 2), k_1–k_4 are the same as for eq 1; $k_5 = 4.48 \times 10^7$; $k_6 = 2.53 \times 10^2$; $k_7 = 7.7 \times 10^1$; $k_8 = 1.54 \times 10^4$; E4 $k_1 = 6.6 \times 10^5$; $k_2 = 4.53 \times 10^1$; $k_3 = 1.63 \times 10^1$; $k_4 = 5.0 \times 10^6$; $k_5 = 4.58 \times 10^6$; $k_6 = 6.0 \times 10^1$; $k_7 = 1.7 \times 10^1$; $k_8 = 1.66 \times 10^5$; E5 (eq 6): $k_1 = 1.07 \times 10^7$; $k_2 = 2.33 \times 10^2$; $k_3 = 1.9 \times 10^3$; $k_4 = 5.6 \times 10^7$; $k_5 = 6.41 \times 10^6$; $k_6 = 7.0 \times 10^1$; (eq 4): $k_1 = 8.47 \times 10^6$; $k_2 = 1.51 \times 10^2$; $k_3 = 1.17 \times 10^2$; $k_4 = 1.35 \times 10^7$; k_5, 6.41×10^6; $k_6 = 7.0 \times 10^1$; E6, $k_1 = 3.7 \times 10^7$; $k_2 = 1.32 \times 10^3$; $k_3 = 1.46 \times 10^4$; $k_4 = 1.9 \times 10^7$; E7, $k_1 = 3.98 \times 10^7$; $k_2 = 1.29 \times 10^3$; $k_3 = 1.55 \times 10^3$; $k_4 = 1.56 \times 10^7$. The enzyme concentrations were (mol L^{-1}): E1, 2.96×10^{-6}; E2, 6.9×10^{-5}; E3, 1.66×10^{-7}; E4, 1.09×10^{-6}, E5, 2.6×10^{-7}; E6, 7.96×10^{-7}; E7, 1.40×10^{-7}.

RESULTS

Comparison of the ^{31}P NMR time courses for samples in which transketolase activity was reduced to 56% of its normal value (Figure 3A) with the control (Figure 3B) shows that flux through the nonoxidative portion of the pentose phosphate pathway was reduced considerably (to 44%) in the sample with diminished transketolase activity. The concentration of the pentose phosphates (ribose 5-phosphate, xylulose 5-phosphate, and ribulose 5-phosphate) remained high, even at the end of the time course, and glucose 6-phosphate accumulated only to a small extent. The rate of appearance of sedoheptulose 7-phosphate and dihydroxyacetone phosphate (which was in equilibrium with glyceraldehyde 3-phosphate due to the activity of triosephosphate isomerase) was retarded.

Measurements of the peak areas (integrals) of the α- and β-anomers of glucose 6-phosphate in the NMR time courses enabled the calculation of a linear rate of glucose 6-phosphate formation (between approximately 18 and 32 min for 100% hematocrit equivalent) in a normal hemolysate of 0.14 mmol (L of red blood cells)$^{-1}$ min^{-1} (n = 4). Flux through the transketolase reaction with ribose 5-phosphate and xylulose 5-phosphate as substrates was estimated to be 0.32 mmol (L of red blood cells)$^{-1}$ min^{-1}, and flux through the transaldolase reaction was estimated to be 0.45 mmol (L of red blood cells)$^{-1}$ min^{-1}. These values were based on enzyme activities of 320 units (L of red blood cells)$^{-1}$ for transketolase and 1408 units (L of red blood cells)$^{-1}$ for transaldolase. The transaldolase

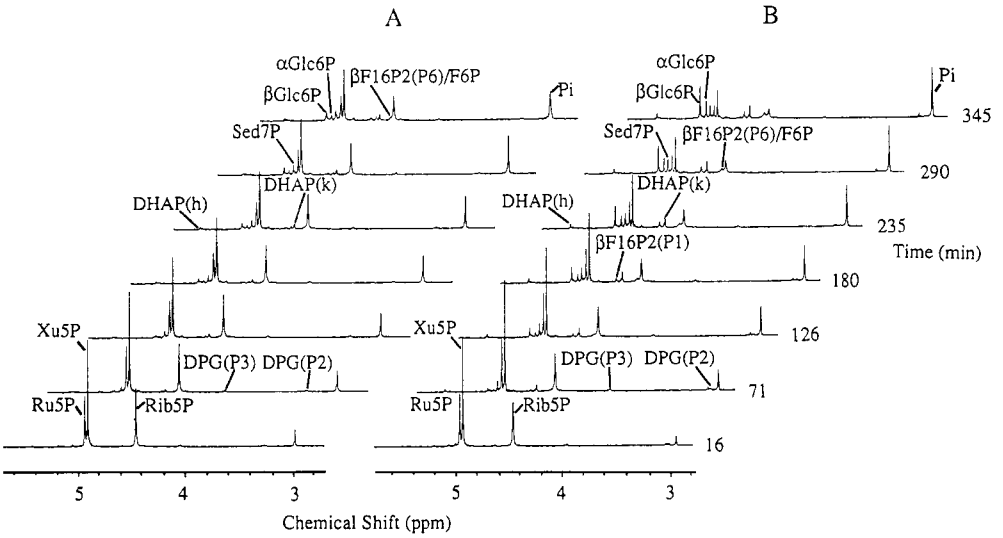

FIGURE 3: Time courses of ^{31}P NMR spectra obtained after the addition of hemolysate with 56% of its normal transketolase activity (A) and with normal activity (B) to a sample containing ribose 5-phosphate at 37 °C. The phosphorus atom from which the resonance arises is given in brackets after the substrate, where necessary, for clarity. The sample composition and NMR conditions are given under Materials and Methods; see also the abbreviations footnote.

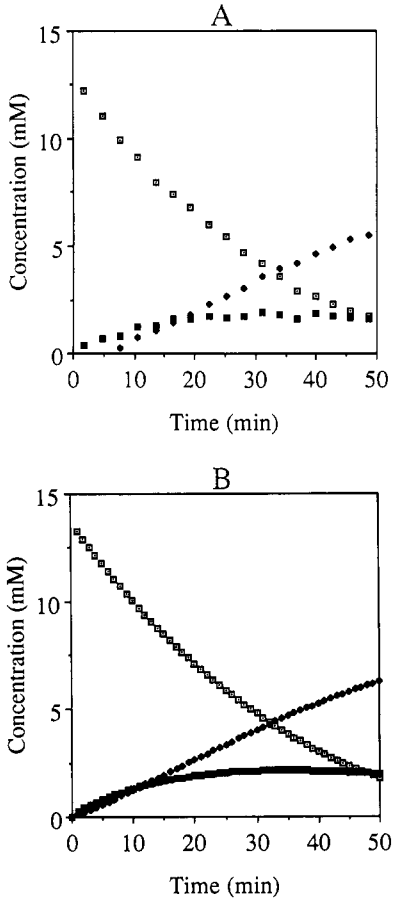

FIGURE 4: Time courses of major metabolites derived from ^{31}P NMR spectra (A) and from computer model simulations using normal enzyme concentrations (B). The symbols denote the following: (■) sedoheptulose 7-phosphate; (◆) glucose 6-phosphate; (⊡) xylulose 5-phosphate + ribulose 5-phosphate.

activity was measured in the reverse direction and divided by 3.1 (this is the ratio of the reported V_{max} values for the forward and reverse reactions) to obtain a value of flux in the forward direction (Srivastava & Hübscher, 1966).

The concentrations of sedoheptulose 7-phosphate, glucose 6-phosphate, and ribulose 5-phosphate and xylulose 5-phosphate, determined by use of NMR, are compared with the results of the simulation using the computer model with normal transketolase activities (Figure 4). These metabolites were chosen because they may readily be quantified from the ^{31}P NMR spectra and are suitable indicators for the kinetic characterization of the nonoxidative pentose phosphate pathway. Computer simulations with transketolase activity modulated to the same values as those used for the NMR time courses were carried out, and plots of metabolite flux versus transketolase activity (Figure 5) reflect the near linear relationship obtained experimentally. Control coefficients for each enzyme were calculated by the phenomenological fitting of a polynomial of degree 4 to the flux versus enzyme activity curves obtained from the computer simulations. Measurement of the slope of this curve at normal enzyme activities yielded flux-control coefficients of 0.74 for transketolase and 0.03 for transaldolase.

To complement our studies of the control exerted by transketolase on the nonoxidative pentose phosphate pathway, a series of experiments in which the transaldolase activity of hemolysates was varied was carried out. It is evident from the ^{31}P NMR time course in which transaldolase was reduced to 54% of the control activity (Figure 6) that the effect on the overall flux through the pathway was not as great as with a similar reduction in transketolase concentration (Figure 3). However, with transaldolase inactivated by 100% in a hemolysate, flux through the pathway was completely eliminated: no glucose 6-phosphate was formed. During this time course, the concentration of sedoheptulose 7-phosphate reaches high levels, becoming the predominant metabolite present in the sample after 75 min (27 min, 100% hematocrit equivalent). Spectrophotometric measurements verified that, upon heating the hemolysates, complete inactivation of transaldolase had occurred and that the transketolase activity of the hemolysate was unaffected by the transaldolase-inactivation procedure.

The low degree of control exerted by transaldolase on flux through the nonoxidative pentose phosphate pathway, at physiological enzyme activities, was demonstrated by the small gradient of the metabolite flux versus transaldolase activity graphs at normal transaldolase concentrations (Figure 5D,E). Because of the limited data (eight points) in the transaldolase-depleted hemolysate, an estimation of the rate of glucose 6-phosphate accumulation was subject to a greater uncertainty than in the other time courses containing more points which

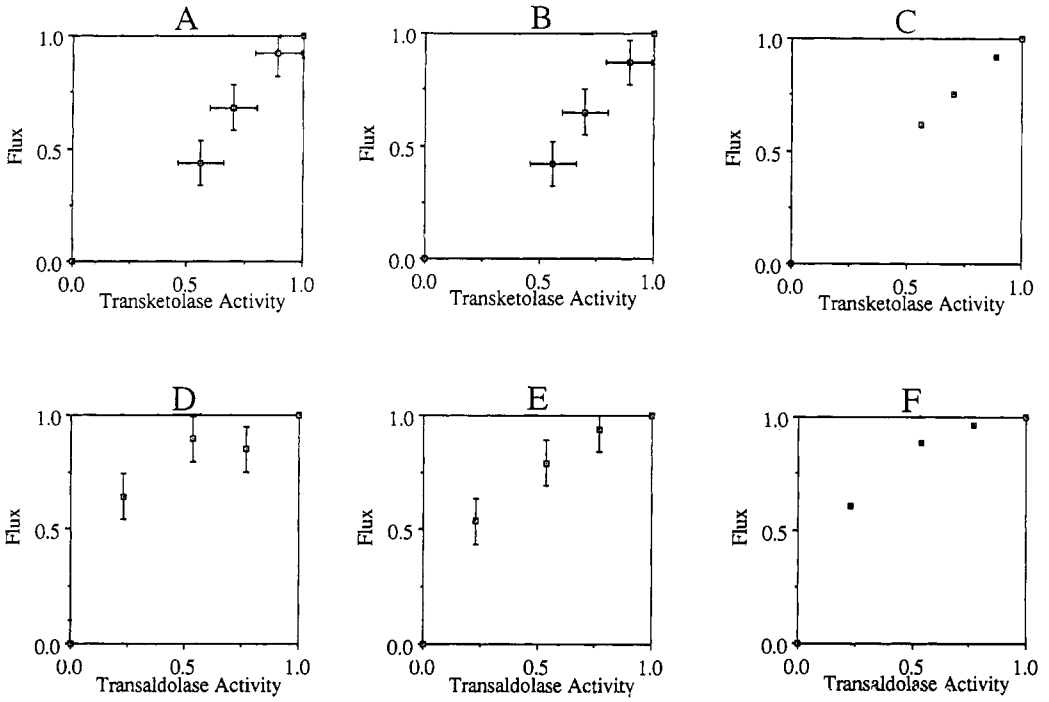

FIGURE 5: Plots showing the dependence of flux through the nonoxidative pentose phosphate pathway on transketolase (A–C) and transaldolase (D–F) activities. An estimate of flux was obtained by measuring the ratio of the linear rate of glucose 6-phosphate formation (between 20 and 30 min for 100% hematocrit equivalent) in the enzyme-depleted samples to that measured in the control samples (A and D) or by taking the ratio of glucose 6-phosphate formed at time = 30 min (100% hematocrit equivalent) in these samples (B and E). Flux was estimated from the computer-simulated time courses (C and F) by measuring the ratio of the linear rate of formation of glucose 6-phosphate when the enzyme activity was set to a reduced value to that obtained when the activity was at its normal value. The error bars on experimental data graphs are arbitrarily set to indicate a ±10% coefficient of variation; in the case of the flux rate, the uncertainty estimate encompasses the error associated with quantifying NMR spectra, and this is commonly <10%, while the errors in spectrophotometric assays, and hence activity estimates, are usually in the range 5–10%. The transaldolase-depleted hemolysates were prepared by mixing normal and inactivated lysates in appropriate proportions, and the error associated with this procedure is likely to be very small and hence no error bars are shown.

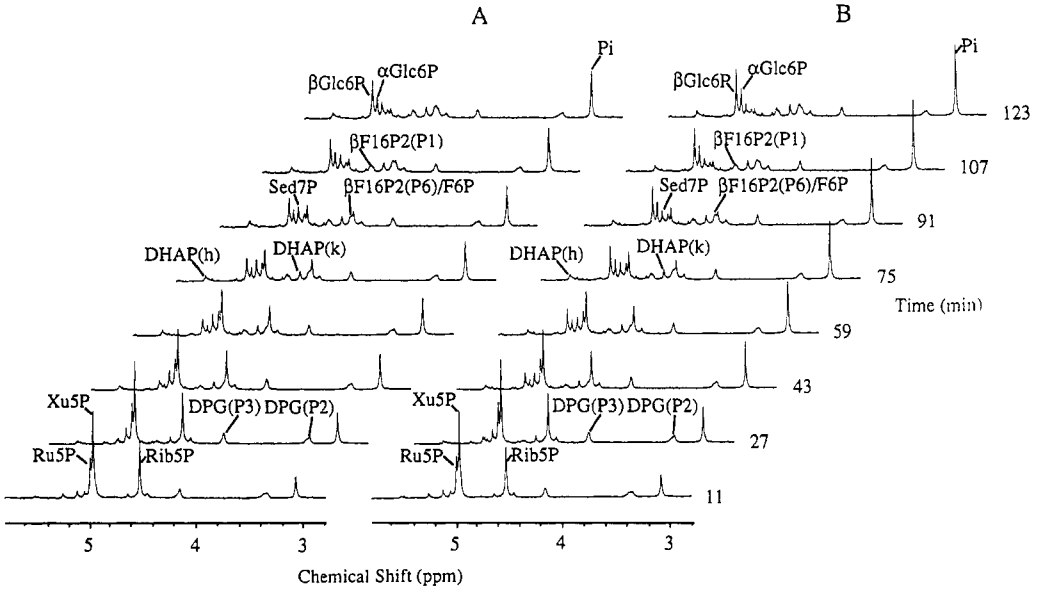

FIGURE 6: Time courses of ^{31}P spectra obtained after the addition of hemolysate with 54% of its normal transaldolase activity (A) and with normal activity (B) to samples containing ribose 5-phosphate at 37 °C. The phosphorus atom from which the resonance arises is given in brackets after the substrate where necessary for clarity. The sample composition and NMR conditions are given under Materials and Methods; see also the abbreviations footnote.

was reflected in the scatter of the points in the flux diagram (Figure 5D).

DISCUSSION

Our experimental results demonstrate the controlling behavior of transketolase in the nonoxidative pentose phosphate pathway as well as the essential role of transaldolase for the operation of the pathway. While the flux curves obtained from the computer simulations (Figure 5C,F) show the high sensitivity of pathway flux to transketolase relative to transaldolase observed experimentally, the magnitudes of flux in transketolase-inactivated hemolysates were found to be lower experimentally than the values predicted by the computer model (Figure 5A–C).

Adjustment of the parameters of the computer model to yield simulations which more closely fitted the NMR data (Figure 4) entailed increasing the concentrations of transaldolase and transketolase by a factor of 1.2 above the spectrophotometrically determined values as well as the alteration of some of the rate constants of these enzymes. Specifically, reducing the K_m of G3P for transaldolase to 8.2 μM was found to lower the levels of Sed7P in the simulations, bringing about a closer match with the experimental data. The reported K_m (Horecker & Smyrniotis, 1955), determined with Brewer's yeast enzyme, is 0.22 mM. The transketolase K_m values for Rib5P and Xu5P were set to 0.63 and 0.30 mM, which are in the vicinity of values recently reported for human erythrocyte enzymes (Booth, 1992). Because of the central importance of the equilibrium constants in the computer model, the literature values (Casazza & Veech, 1986) for the initial transketolase and transaldolase reactions were verified experimentally with ^{31}P NMR using commercially available enzymes. The equilibrium constants for aldolase with F16P2 and Sed17P2 were determined to be 1.7×10^{-5} and 5.3×10^{-6} M, respectively, with ^{31}P NMR, and these values were incorporated into the model. While the set of rate constants chosen represents the best match to experimental data we were able to achieve, the simulations of the computer model showed glucose 6-phosphate reaching a higher level than was observed experimentally (Figure 4). Evidence of the hydrolysis of intermediates of the pathway was apparent in the growth of the inorganic phosphate peak throughout the ^{31}P NMR time courses (Figures 3 and 6), which could only partially be accounted for by the degradation of DPG. The inclusion of phosphatase activity into our computer model may result in an improvement in the ability of the model to simulate the experimental results.

The inhibition of transketolase by 5-phosphoribosyl-1-pyrophosphate (PRPP) and adenosine diphosphate (ADP) has been reported, with the postulate that these inhibitors may be important in controlling the synthesis of nucleotides via their effect on pentose phosphate pathway flux through transketolase (Hosomi et al., 1989). The K_i for PRPP, however, is reported to be 0.14 mM, several orders of magnitude higher than normal levels in the human erythrocyte (Petersen & Quistorff, 1990) so that while ADP is likely to play a regulatory role, it is unlikely that PRPP would be an effector in normal red blood cells.

While NMR is a relatively insensitive technique, it is particularly well-suited to metabolic studies because it offers the advantages of continuous and simultaneous monitoring of a range of metabolites. The measurement of metabolite concentrations is direct, obviating the potential errors associated with extensive sample manipulation. The considerable sensitivity of the chemical shift of ^{31}P metabolites to changes in pH (Robitaille et al., 1991), however, necessitates the confirmation of assignments by the addition of authentic compounds to deproteinized samples. The nuclear Overhauser enhancements, consequent upon broad-band irradiation, were within the range of 0.21–0.25 for all sugar phosphates measured (Glc6P, F6P, DHAP, F16P2, Rib5P, and Xu5P). The longitudinal relaxation times (T_1s), determined for several sugar phosphates were within the range 2.1–3.3 s; additionally, no difference in the intensities of sugar phosphate resonances in a mixture obtained followed incubation of Rib5P with hemolysates was observed when 20-s relaxation delay times were replaced with 90-s delay times.

The specificity of the antitransketolase serum was very high but not absolute (Figure 1). The undiluted serum yielded single, identical reaction lines in an Ouchterlony diffusion gel, when reacted against a crude erythrocyte hemolysate and against various dilutions of highly purified human erythrocyte transketolase. The much more sensitive enhanced chemiluminescence method of detecting membrane-bound antibodies demonstrated a reaction complex with purified transketolase after dilution of the serum 50 000-fold. At this concentration and at 10 000-fold dilution, the serum reacted with only one component of the crude erythrocyte hemolysate, and that had a subunit relative molecular size identical with that of transketolase. At dilutions of 2000-fold (Figure 1) and lower, it was possible to detect weak serum reactions with up to five other components of the hemolysate, all unidentified. Its reaction with a crude extract of *E. coli* was stronger, consistent with immunization of the rabbit by colonic bacteria (Figure 1). The accumulation of intermediates (e.g., Figure 3) in experimental mixtures containing the serum gave no evidence for decreased activity of any of the enzymes of the tested pathway (Figure 2) apart from that of transketolase. While heating at 55 °C inactivates transaldolase, this procedure does not affect holotransketolase (Takeuchi et al., 1984; McIntyre et al., 1989). Moreover, the accumulation of intermediates in heated experimental mixtures gave no evidence for decreased activity of any of the enzymes of the tested pathway apart from that of transaldolase.

There is evidence that transketolase and transaldolase may be bound in a complex in some cells (Kiely et al., 1969; Wood, 1981). A termolecular complex of transketolase, transaldolase, and glyceraldehyde-3-phosphate dehydrogenase has also been proposed for *Candida utilis* (Wood et al., 1985). The existence of such complexes could mean that the kinetic data obtained from the individual enzymes may be far from reflecting their activity in vivo. If ternary complexes are formed in vivo, they should be fully represented, in a kinetic sense, in the hemolysate, except that they may dissociate upon dilution. However, it should be said that purification of transketolase by "gentle" chromatographic procedures readily separates it from the other two enzymes, although this was not achieved by Heinrich and Wiss (1971). A possible reason for the putative evidence for these complexes is a similarity of the molecular weights of each of the enzymes and the dependence of previous workers on gel filtration for purification of transketolase. Furthermore, the rates of Glc6P accumulation determined from the NMR time-course experiments using two different hemolysate concentrations (0.10 for the transketolase control experiments and 0.36 for the transaldolase control) were the same within experimental error, indicating that if enzyme complexes do form, their perturbation of the in vitro kinetics is small.

CONCLUSIONS

In conclusion, we have demonstrated the high flux-control coefficient of transketolase in the nonoxidative pentose phosphate pathway of human erythrocytes. This was achieved using hemolysates and selective inhibition of transketolase with antiserum. The reactions of the pentose phosphate pathway were monitored simultaneously by ^{31}P NMR spectroscopy. The essential role of transaldolase in the operation of the pathway was demonstrated, and the experimental data were comprehensively described with a computer model of the classical type pentose phosphate pathway, although good fits to the data were obtained only after some of the equilibrium constant and K_m values had been varied from the reported values.

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Registry No. Glc6P, 56-73-5; F6P, 643-13-0; DHAP, 57-04-5; F16P2, 488-69-7; Rib5P, 4300-28-1; Xu5P, 4212-65-1; transketolase, 9014-48-6; transaldolase, 9014-46-4.