Biochem. J. (1991) 273, 587–592 (Printed in Great Britain)

Properties of human testis-specific lactate dehydrogenase expressed from *Escherichia coli*

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The cDNA encoding the C_4 isoenzyme of lactate dehydrogenase (LDH-C_4) was engineered for expression in *Escherichia coli*. The *Ldh-c* open reading frame was constructed as a cassette for production of the native protein. The modified *Ldh-c* cDNA was subcloned into the prokaryotic expression vector pKK223-3. Transformed *E. coli* cells were grown to mid-exponential phase, and induced with isopropyl β-D-thiogalactopyranoside for positive regulation of the *tac* promoter. Induced cells expressed the 35 kDa subunit, which spontaneously formed the enzymically active 140 kDa tetramer. Human LDH-C_4 was purified over 200-fold from litre cultures of cells by AMP and oxamate affinity chromatography to a specific activity of 106 units/mg. The enzyme was inhibited by pyruvate concentrations above 0.3 mM, had a K_m for pyruvate of 0.03 mM, a turnover number (nmol of NADH oxidized/mol of LDH-C_4 per min at 25 °C) of 14000 and was heat-stable.

INTRODUCTION

Testis-specific lactate dehydrogenase (LDH-C_4) from the mouse has been purified, crystallized and characterized biochemically (reviewed in Goldberg, 1987*a*). Different kinetic properties of the testicular and somatic isoenzymes of LDH have been attributed to amino acid sequence differences, especially in the highly conserved coenzyme-binding domain (Musick & Rossmann, 1979). The putative amino acid sequence of human LDH-C_4 deduced from a cDNA clone (Millan *et al.*, 1987) was found to be dissimilar to active-centre residues of mouse LDH-C_4 (Li *et al.*, 1983), implying that generalization concerning the biochemical properties of the testis-specific isoenzymes might not be applicable. In order to obtain sufficient quantities of human LDH-C_4 for further investigation, and because of the impracticality of purifying the protein from healthy human testes, we have used recombinant DNA technologies to provide a source of human LDH-C_4.

The present paper describes the engineering of the full-length human *Ldh-c* cDNA clone for over-expression of human LDH-C_4 from bacteria. The human protein was produced as a catalytically active tetramer of 140 kDa and purified to homogeneity by two-step affinity chromatography. The kinetics of pyruvate reduction by human LDH-C_4 from *E. coli* were compared with those for mouse LDH-C_4 as well as with those for the somatic isoenzymes of LDH. In addition, we have demonstrated that human LDH-C_4 has heat-stability properties similar to those of the mouse isoenzyme (Hawtrey & Goldberg, 1970).

MATERIALS AND METHODS

Engineering of human *Ldh-c* cDNA for expression

The human *Ldh-c* cDNA was cloned by screening a λgt11 human testis cDNA expression library with rabbit antisera and monoclonal antibodies against mouse LDH-C_4 (Millan *et al.*, 1987). The entire 1523 bp human *Ldh-c* cDNA fragment was isolated from the λ clone by a partial *Eco*RI digestion, separated by agarose-gel electrophoresis, transferred to a DEAE-cellulose membrane (Schleicher and Schuell), recovered by high-salt elution and purified by ethanol precipitation (Dretzen *et al.*, 1981). A 1250 ng portion of the 1.5 kbp fragment was ligated into 500 ng of a 2.9 kbp vector (a 5-fold molar excess of fragment over vector), an *Eco*RI digested and phosphatase-treated pGEM-3 plasmid (Promega). The HB101 strain of *E. coli* was transformed with 100 ng of the ligation mixture, and colonies containing recombinant plasmids were selected on Luria–Bertani (LB) medium plates with 50 μg of ampicillin/ml. Plasmid DNA from the transformants was isolated by the small-scale alkaline-lysis method, digested with restriction enzymes, electrophoresed through a 1% agarose gel containing 0.5 μg of ethidium bromide/ml and detected by photographing the gel under u.v. light. Three out of nine transformants contained the insert in the correct orientation. One was prepared by the large-scale alkaline-lysis method, purified by centrifugation through a CsCl equilibrium gradient, desalted by ethanol precipitation and designated pG3HC 1.5. Molecular-biology procedures were adapted from standard protocols (Maniatis *et al.*, 1982), and enzymes were purchased from New England Biolabs.

Most of the *Ldh-c* open reading frame (ORF) is contained within a 1390 bp *Hin*cII fragment. Digestion with *Hin*cII removed the 5′ untranslated sequences and the first 5 bp of the ORF. A 5′ linker was constructed with a *Sma*I site adjacent to the first 5 bp of the ORF by synthesizing two oligomers with the following complementary sequences:

CCCGGGATGTC
GGGCCCTACAG

Oligonucleotides were synthesized by the phosphoramidite method on an Applied Biosystems synthesizer, and purified on a Waters Delta-Pak C_{18} (30 nm-pore-size; 5 μm-particle-size) column by reverse-phase h.p.l.c. The latter oligomer only was kinased to increase the chances of only one linker attaching and in the proper orientation. The two oligomers were boiled for 5 min, annealed by slow cooling to room temperature, and ligated with a 100:1 linker/*Ldh-c* fragment ratio. The regenerated

Abbreviations used: LDH, lactate dehydrogenase; ORF, open reading frame.

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ORF was digested with *Dra*I, which removed the AA of the stop codon TAA, and all of the 3′ untranslated sequences. An oligomer was synthesized with the palindromic sequence:

AAACCCGGGTTT

treated with kinase, annealed to itself and ligated. Tandem repeats of the 5′ and 3′ linkers were removed by digestion with *Xma*I. The *Ldh-c* ORF engineered with *Xma*I (*Sma*I) linkers was subcloned by the above methods into the *Xma*I site of pGEM-4 (Promega) to construct pHum*Ldh-c* ORF (Fig. 1).

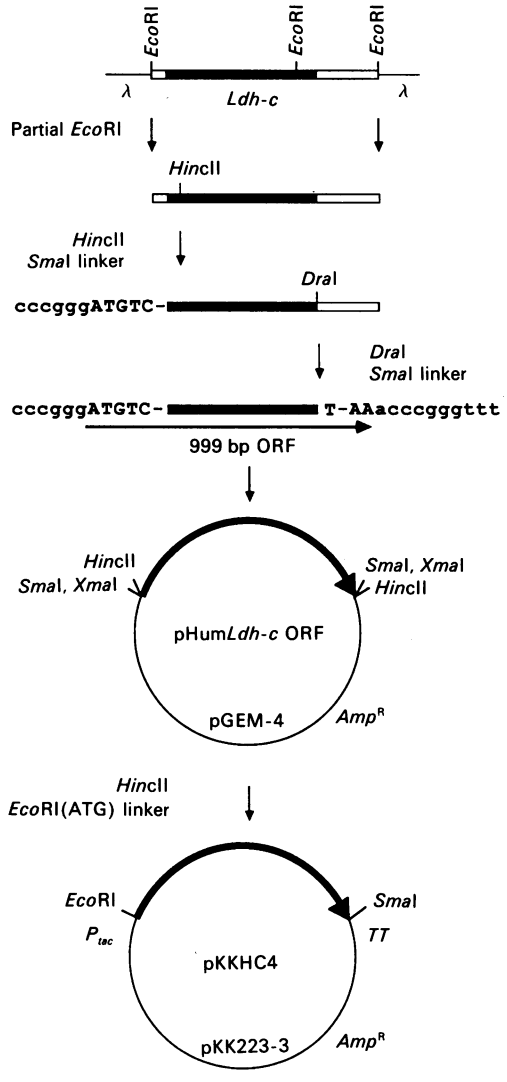

Fig. 1. Genetic engineering scheme for construction of pHum*Ldh-c* ORF and pKKHC4

The black bar denotes the human *Ldh-c* cDNA contained within the λgtll vector (Millan *et al.*, 1987). Both the pGEM-4 and pKK223-3 vectors are derived from pBR332, a multiple-copy-number plasmid (Twigg & Sherratt, 1980) that confers ampicillin-resistance. pKK223-3 allows expression of human LDH-C_4 by induction of the hybrid *tac* promoter. The *tac* promoter consists of the −35 conserved TTGACA sequence from the *trp* promoter, and the −10 conserved TATAAT sequence, the operator and the Shine–Dalgarno sequence comprising the ribosome-binding site from the *lac* UV-5 promoter (de Boer *et al.*, 1983; Amann *et al.*, 1983). The human *Ldh-c* ORF was inserted asymmetrically into the *Eco*RI and *Sma*I sites of the M13mp8 polylinker (Vieira & Messing, 1982), which is followed by the strong *rrnB* transcription terminators (Brosius *et al.*, 1981*a*,*b*). See the Materials and methods section for cloning details.

The *Ldh-c* ORF was modified for subcloning into the plasmid expression vector pKK223–3 (Pharmacia). The *Hin*cII fragment containing all but the first 5 bp of the *Ldh-c* ORF was isolated from pHum*Ldh-c* ORF. However, the 5′ and 3′ untranslated sequences were already removed, and the stop codon and 3′ *Sma*I remained intact since *Hin*cII cuts downstream of *Sma*I in the multiple cloning site of pGEM-4. The *Ldh-c* natural *Eco*RI site was methylated with *Eco*RI methylase. Two oligomers were synthesized with the complementary sequences:

GAATTCATGTC
CTTAAGTACAG

and purified. Again, the latter oligomer only was treated with kinase, and the two oligomers were annealed and ligated with a 100:1 linker/*Ldh-c* fragment ratio. Tandem repeats of 5′ and 3′ linkers were removed by digestion with *Eco*RI and *Xma*I. The *Ldh-c* ORF fragment was subcloned by the above methods into an *Eco*RI- and *Xma*I-digested pKK223-3 vector and designated pKKHC4 (Fig. 1).

Over-expression and purification of human LDH-C_4

Cell growth and induction. The W3110 *lacI*^{q} strain of *E. coli* cells transformed with pKKHC4 were grown in 1-litre volumes of Luria–Bertani medium with ampicillin (50 μg/ml) at 37 °C. Cells were grown to mid-exponential phase ($A_{600} = 0.5$) and induced with 1 mM-isopropyl β-D-thiogalactopyranoside. For negative controls, parallel cultures were grown of transformed cells that were not induced and of non-transformed host cells that were not induced. All cultures were incubated for an additional 2 h beyond mid-exponential phase. Cells were harvested at 5000 *g* for 30 min at 4 °C, and pellets were frozen at −20 °C.

Cell lysis and protein fractionation. Frozen pellets were combined from 20-litre cultures (60 g wet wt. of cells), thawed quickly at 37 °C, resuspended in 200 ml of 0.1 M-sodium phosphate buffer, pH 7.0 containing 1 mM-EDTA and 1 mM-dithiothreitol and sonicated at 4 °C until the cells were lysed. The crude extract was adjusted to 33% saturated with solid $(NH_4)_2SO_4$ (196 g/l), and after stirring for 1 h at 4 °C the precipitated protein was removed by centrifugation at 12100 *g* for 30 min at 4 °C. The supernatant was adjusted to 55% saturation with $(NH_4)_2SO_4$ (351 g/l) and stirred overnight at 4 °C. The precipitate was collected by centrifugation at 12100 *g* for 30 min at 4 °C, and resuspended in a minimum volume of 0.1 M-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 1 mM-dithiothreitol. The protein was then heated for 15 min at 55 °C, cooled and centrifuged at 12100 *g* for 30 min, and dialysed against 0.1 M-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 1 mM-dithiothreitol at 4 °C.

AMP–agarose chromatography. The dialysed cell extract was loaded at a flow rate of 1 ml/min on to a 40 ml volume column of 5′-AMP–agarose (Pharmacia) equilibrated with 0.1 M-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 1 mM-dithiothreitol, and washed through with the same buffer until the A_{280} of the eluate fell to baseline (Lee *et al.*, 1982). LDH was specifically eluted with a reduced NAD^+–pyruvate adduct (50 mg of NAD^+ and 77 mg of sodium pyruvate in 100 ml of 0.1 M-sodium phosphate buffer, pH 7.0). Eluate fractions with peak LDH activity were pooled, concentrated by ultrafiltration and dialysed against 0.02 M-sodium phosphate buffer, pH 6.8, containing 1 mM-EDTA and 1 mM-dithiothreitol.

Oxamate–agarose chromatography. The dialysed protein was

loaded on to a 14 ml-volume oxamate–agarose (Sigma Chemical Co.) column equilibrated with 0.02 M-sodium phosphate buffer, pH 6.8, containing 0.2 M-NaCl and 200 μM-NADH (O'Carra & Barry, 1972; Spielmann *et al.*, 1973; O'Carra *et al.*, 1974; Kolk *et al.*, 1978) and washed with the same buffer until no more protein was detected by the Bio-Rad micro-assay. LDH-C_4 was eluted with 10 mM-pyruvate in 0.02 M-sodium phosphate buffer, pH 6.8, containing 0.2 M-NaCl. The 10 ml fraction with the highest LDH activity was concentrated by ultrafiltration and dialysed against 0.1 M-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 1 mM-dithiothreitol.

Protein analysis. Each step in the protein purification was assayed for total protein (Bio-Rad standard or micro-assay) and LDH activity (see below). Catalytic activity of the over-expressed human LDH-C_4 was also demonstrated by PAGE on native 7.5% polyacrylamide gels stained for LDH activity (Goldberg, 1964). Degree of purity of human LDH-C subunits was shown by SDS/12%-PAGE gels stained with Coomassie Brilliant Blue (Laemmli, 1970).

Kinetic studies

LDH enzyme activity was routinely measured spectrophotometrically by the decrease in absorbance at 340 nm due to NADH oxidation (Hawtrey & Goldberg, 1970) in 3 ml of 0.5 M-sodium phosphate buffer, pH 7.0, containing 0.15 mM-NADH and 0.25 mM-pyruvate at 25 °C. To detect specifically human L-LDH activity and not *E. coli* D-LDH activity, lactate oxidation was measured spectrophotometrically by the increase in absorbance at 340 nm due to NAD^+ reduction (Neilands, 1955) in 0.1 M-Tris/HCl buffer, pH 8.4, containing 1 mM-NAD^+ and 24 mM-DL-lactate. One unit of enzyme activity is defined as the amount of LDH that oxidizes 1 μmol of NADH/min. The K_m for pyruvate was calculated from a Lineweaver–Burk plot.

Heat-stability assays were performed by diluting the pure enzyme in 0.1 M-sodium phosphate buffer, pH 7.0, containing 0.1% BSA, incubating for 10 min at temperatures increasing from 0 °C, to 85 °C, cooling and measuring activity spectrophotometrically.

The turnover number is defined as the number of mol of NADH oxidized/mol of human LDH-C_4 per min at 25 °C. The activity was measured spectrophotometrically as above, and converted by using a molar absorption coefficient of 6.2×10^3 $M^{-1} \cdot cm^{-1}$ for NADH (Horecker & Kornberg, 1948) and a molecular mass of 140000 Da for LDH-C_4.

RESULTS

The human *Ldh-c* cDNA (1523 bp) contains 66 bp of 5′ untranslated sequences, a 999 bp ORF including the stop codon and 130 bp of 3′ untranslated sequences (Millan *et al.*, 1987) followed by 328 bp of unidentified DNA, probably an artifact of the cDNA cloning. The cDNA was modified by removing the 5′ untranslated sequences by digestion with *Hin*cII, and the 3′ untranslated sequences by digestion with *Dra*I (Fig. 1). The 5′ linker added an *Sma*I site and replaced the start codon and half of the *Hin*cII site. Regeneration of the *Hin*cII site implied that the linker and start codon were engineered in the correct orientation and reading frame. The 3′ linker replaced the stop codon, the *Dra*I site and added an *Sma*I site. Regeneration of the *Dra*I site implied that the linker was ligated on properly and the stop codon was reconstructed in the correct reading frame. Two of 18 clones contained the engineered *Ldh-c* cDNA ORF bounded by *Sma*I linkers in pGEM-4. This construction was designated pHum*Ldh-c* ORF.

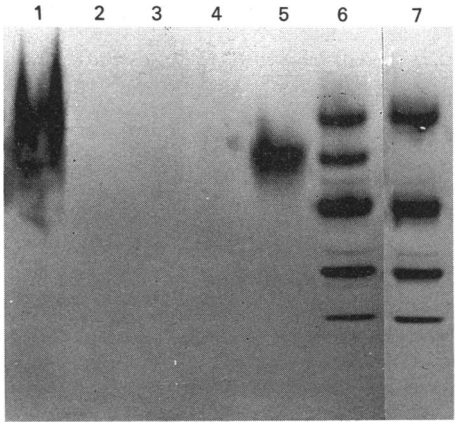

Fig. 2. Demonstration of LDH-C_4 enzymic activity in *E. coli*

Parallel cultures of W3110 *lacI*^{q} cells transformed with pKKHC4 were grown to mid-exponential phase, and induced with isopropyl β-D-thiogalactopyranoside for expression of human LDH-C_4 (lane 1), or not induced (lane 2), or induced and blocked with antiserum to mouse LDH-C_4 (lane 3). Non-transformed W3110 *lacI*^{q} host cells were grown and not induced (lane 4). Cell lysates were analysed by PAGE on a native 7.5% polyacrylamide gel, stained for LDH activity with lactate as the substrate. Purified human LDH-C_4 (lane 5) was electrophoresed along with human testis extract (lane 6) and human testis extract blocked with antiserum to mouse LDH-C_4 (lane 7) for controls. The testis extract (lanes 6 and 7) also contains LDH isoenzymes consisting of the A- and B-subunit-containing heterotetramers from the anode (bottom of gel) to the cathode as follows: LDH-B_4, LDH-B_3A, LDH-B_2A_2, LDH-C_4 (lane 6 only) and LDH-BA_3. In addition, faintly staining sub-bands can be seen in these extracts.

The cDNA was then engineered for expression of human LDH-C_4 from bacteria. The *Hin*cII fragment was isolated from pHum*Ldh-c* ORF and a 5′ linker was attached to add an *Eco*RI site, the start codon and half the *Hin*cII site (Fig. 1). Again, regeneration of the *Hin*cII site implied correct construction. Five out of 18 clones contained the *Ldh-c* ORF bounded by 5′ *Eco*RI and 3′ *Sma*I linkers in pKK223-3, and were designated pKKHC4.

E. coli cells transformed with pKKHC4 were grown to mid-exponential phase, induced with isopropyl β-D-thiogalactopyranoside and lysed by sonication. Synthesis of human LDH-C_4 in these cells was shown by non-denaturing PAGE. Native gels stained for LDH activity demonstrated that induced cells express LDH as an enzymically active tetramer (Fig. 2). The crude LDH-C_4 migrated as a diffuse zone of activity near the LDH-C_4 band in the human testis control. Non-induced cells and host cells containing no plasmid had no LDH activity, as expected, since *E. coli* do not contain L-LDH (Tarmy & Kaplan, 1968*b*; Everse & Kaplan, 1973). Therefore only cells transformed with the human *Ldh-c* sequence under control of the *tac* promoter, and induced with isopropyl β-D-galactopyranoside, expressed LDH-C_4. When the cell lysate was incubated with rabbit anti-(mouse LDH-C_4) serum before electrophoresis, migration of the LDH-C_4 was specifically blocked. The remaining activity was due to heterotetramers of LDH-A and -B subunits. The purified human LDH-C_4 co-migrated with the LDH-C_4 band from human testis.

Human LDH-C_4 was purified over 200-fold from bacteria with a final specific activity of 106 units/mg of protein, and a yield of 13% (Table 1). Lactate oxidation, which was measured to detect specifically L-LDH activity, was consistently about one-quarter the pyruvate reduction activity, with a final specific activity of 26 units/mg (LeVan, 1989).

The degree of purity was analysed initially by SDS/PAGE

Table 1. Summary of human LDH-C_4 purification from *E. coli*

For experimental details see the Materials and methods section.

Sample	Total LDH-C_4 activity (units)	Total protein (mg)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Crude lysate	4108	7740	0.5	1	100
33–55%-satn.-$(NH_4)_2SO_4$ fraction	2736	1083	2.5	5	67
AMP–agarose eluate	2273	57	39.9	80	55
Oxamate–agarose eluate	530	5	106.0	212	13

(Fig. 3). The crude lysate contained no predominant band around 35 kDa, which indicated that the subunit was not over-expressed as a major cellular protein. Thus excess LDH-C subunits had not denatured to form inclusion bodies. A band seen in the total cell lysate was absent from the run-through of the AMP–agarose column. The eluate from the AMP–agarose column contained a predominant band of the same mobility and a few contaminants. The eluate from the oxamate–agarose column demonstrated that the protein was purified to homogeneity, and this band migrated at 35 kDa, as expected for the LDH subunit. Subsequently, the first 20 *N*-terminal residues of the pure protein were sequenced. Serine, as the first amino acid residue, indicated that the initiation methionine residue was cleaved off.

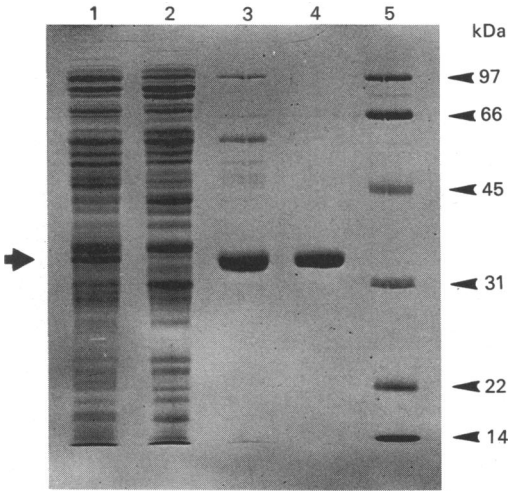

Fig. 3. Purification of human LDH-C_4 from *E. coli*

Litre cultures of W3110 *lacI*^{q} cells, transformed with pKKHC4, were grown and induced with isopropyl β-D-thiogalactopyranoside for expression of human LDH-C_4. Cells were harvested and lysed, protein was precipitated between 33% and 55% saturation with $(NH_4)_2SO_4$, heated at 55 °C for 15 min and dialysed. LDH-C_4 was purified by AMP–agarose and then oxamate–agarose affinity chromatography. Fractions of crude cell lysate (lane 1), AMP wash (lane 2), AMP–agarose eluate (lane 3) and oxamate–agarose eluate (lane 4) with LDH activity were electrophoresed on a SDS 12%-polyacrylamide gel and proteins were detected by staining with Coomassie Blue. The arrow at the left denotes the position of the human LDH-C subunit, and the molecular masses of the protein markers (lane 5) are indicated on the right.

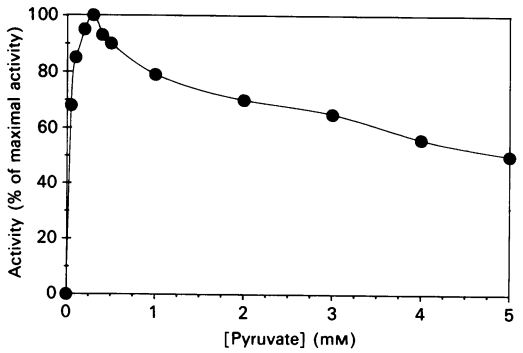

Fig. 4. Pyruvate inhibition of human LDH-C_4 enzymic activity

LDH enzyme activity was measured spectrophotometrically by the decrease in absorbance at 340 nm due to NADH oxidation in 0.05 M-sodium phosphate buffer, pH 7.0, containing 0.15 mM-NADH and increasing concentrations of pyruvate from 0.1 to 5.0 mM at 25 °C.

Maximal LDH-C_4 activity was achieved between 0.25 mM- and 0.30 mM-pyruvate (Fig. 4). LDH-C_4 showed substrate inhibition with increasing concentrations of pyruvate. At 5.0 mM-pyruvate, activity was 50% maximal. The K_m for pyruvate was 0.03 mM. These optimal pyruvate concentration and K_m values are comparable with the respective values of 0.2 mM and 0.053 mM for LDH-C_4 derived from human testes (Svasti & Viriyachai, 1975*b*), 0.62 mM and 0.05 mM for LDH-C_4 derived from human spermatozoa (Wilkinson & Withycombe, 1965), and 0.5 mM and 0.026 mM for LDH-C_4 from mouse testes (Goldberg, 1972). At 1 mM-pyruvate mouse LDH-C_4 activity was 58% maximal (Hawtrey & Goldberg, 1970).

Human LDH-C_4 was stable at temperatures up to 55 °C, and activity subsequently decreased at higher temperatures (Fig. 5). LDH-C_4 maintained at 65 °C was stable for 10 min, with 70% of the activity remaining after 1 h at 65 °C (results not shown).

The turnover number with pyruvate was calculated to be 14000 mol of NADH oxidized per mol of human LDH-C_4 per min. This is higher than the value (2980) for mouse LDH-C_4 (Goldberg, 1972), yet lower than the values (ranging from 41500 to 160000) for the somatic LDH isoenzymes (Pesce *et al.*, 1967).

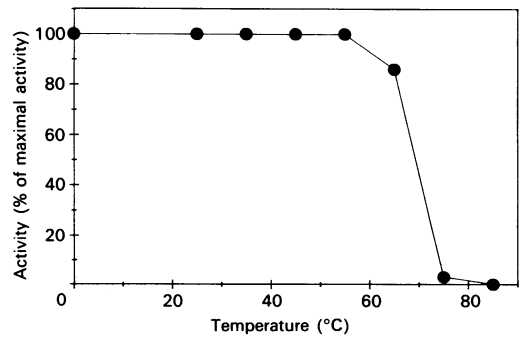

Fig. 5. Heat-stability of purified human LDH-C_4

Heat-stability assays were carried out by diluting the pure enzyme in 0.1 M-sodium phosphate buffer, pH 7.0, containing 0.1% BSA, incubating for 10 min at temperatures increasing from 0 °C to 85 °C, cooling, and measuring LDH activity spectrophotometrically in 0.05 M-sodium phosphate buffer, pH 7.0, containing 0.15 mM-NADH and 0.25 mM-pyruvate at 25 °C.

DISCUSSION

The human *Ldh-c* cDNA was engineered for expression of the catalytically active tetramer in *E. coli* in milligram quantities. Human LDH-C_4 is a 140 kDa protein consisting of four identical subunits of 331 amino acids residues each. A cDNA cassette containing only the LDH-C subunit coding sequences was subcloned into pKK223-3 because this vector allows expression of the native protein. To maximize expression, all the 5′ and 3′ non-coding sequences were removed, because they have the potential to interfere with the regulatory mechanisms of the host expression system. The *Ldh-c* coding sequence including the initiation codon ATG was inserted into a unique cloning site 10 bp past the Shine–Dalgarno sequence (Shine & Dalgarno, 1975) for efficient ribosome-binding (Gold *et al.*, 1981; Stormo *et al.*, 1982; Kozak, 1983). Analysis of the mRNA sequence from the Shine–Dalgarno sequence through the ATG and the cDNA encoding the first 33 amino acid residues indicated no potential for significant mRNA secondary structure (Tinoco *et al.*, 1973). Although the human *Ldh-c* codon usage was not optimal for all amino acids in *E. coli*, the protein was synthesized.

pKK223-3 has been used with some success for over-production of prokaryotic and eukaryotic proteins in *E. coli*. However, replacement of the replication origin of pKK223-3 derived from pBR322 with that of pUC9 produced chicken lysozyme at about 25% of total cellular protein, which was 10-fold more than achieved with the analogous construction in unmodified pKK233-3 (Miki *et al.*, 1987). Also, replacement of the ribosome-binding site of the *tac* promoter with a synthetic sequence containing greater complementarity to the 3′ end of 16 S rRNA produced a fusion protein of β-galactosidase and polyoma T antigen 5–10-fold more than in control cells (Pallas *et al.*, 1986). Thus it may be possible to increase the production of LDH-C_4 further with an improved expression vector.

Purification of human LDH-C_4 from *E. coli* is not complicated by the presence of an endogenous *E. coli* L-LDH. The primitive labile D-LDH contributes to a portion of the initial LDH activity measured spectrophotometrically. This is not detected on the native gel stained for oxidation of lactate because D-LDH is functionally a unidirectional enzyme that acts as a pyruvate reductase (Tarmy & Kaplan, 1968*a*,*b*). Other prokaryotic dehydrogenases with the conserved subunit size of 35 kDa (Kaplan, 1965) would co-migrate with the LDH-C band on the SDS/polyacrylamide denaturing gel, but with their different charges and stabilities are lost during the purification procedure. Also, isolation of LDH-C_4 from bacteria is not complicated by the presence of the eukaryotic LDH-A_4 and LDH-B_4 somatic isoenzymes, as occurs with isolation from human testes (Svasti & Viriyachai, 1975*a*). The yield of human LDH-C_4 purified from *E. coli* is 5 mg per 20 litres of cells, as compared with 4 mg of LDH-C_4 from 225 g of human testes (Svasti & Viriyachai, 1975*a*).

Human LDH-C_4 is heat-stable relative to the somatic LDH isoenzymes (Wilkinson & Withycombe, 1965; McKee *et al.*, 1972), as is mouse LDH-C_4 (Hawtrey & Goldberg, 1970). Similarly to the LDH isoenzymes containing B subunits, human LDH-C_4 is inhibited by higher concentrations of pyruvate (Plagemann *et al.*, 1960; Wilkinson & Withycombe, 1965; Latner *et al.*, 1966). A turnover number intermediate between those of mouse LDH-C_4 and the somatic isoenzymes may be indicative of the greater similarity of the sequence of the coenzyme-binding site of human LDH-C (Millan *et al.*, 1987) to the somatic isoenzymes than to the sequence of mouse LDH-C (Pan *et al.*, 1983). Although the kinetic properties of LDH-C_4 do not suggest a function for the testis-specific isoenzyme, its expression and purification from bacteria has provided us with an abundant source of a human sperm antigen for testing as an immunocontraceptive vaccine. The testis isozyme is predominantly cytosolic; however, a portion of the enzyme is adsorbed on the sperm cell surface (Erickson *et al.*, 1975; Storey & Kayne, 1977; Alvarez & Storey, 1984). Antibodies to LDH-C_4 do not cross-react with the somatic isoenzymes LDH-A_4 and LDH-B_4 (Goldberg, 1971; Liang *et al.*, 1986). Because LDH-C_4 is restricted to the germinal epithelium of testes and to spermatozoa (Blanco & Zinkham, 1963; Goldberg, 1963; Goldberg & Hawtrey, 1967), it would be seen as a foreign protein in the female reproductive tract. These cell-specific properties have allowed LDH-C_4 to be used as a model for development of a contraceptive vaccine by eliciting an immune response directed against sperm (Goldberg, 1987*b*). The antigenic determinants can be mapped, and correlated with the three-dimensional structure derived from X-ray crystallography, as was done with mouse LDH-C_4 (Wheat & Goldberg, 1985*a*,*b*; Wheat *et al.*, 1985; Hogrefe *et al.*, 1987; Hogrefe *et al.*, 1989). Ultimately, human LDH-C_4 in addition to other future characterized sperm antigens may be developed into an effective multivalent immunocontraceptive vaccine.

We are grateful to Dr. Patricia O'Hern and Dr. Thomas Wheat for advice on purification of the protein, and to Dereck Wood for technical assistance. We thank Dr. Emanuel Margoliash and Dr. David Ollis for critical reading of the manuscript, and Dr. Richard Gaber for his creative computer consultation. This work was supported by National Institutes of Health Grants HD05863 and HD23771 (to E. G.).

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Received 20 April 1990/19 June 1990; accepted 11 July 1990