J. Sci. Soc. Thailand 1 (1975), 57–71

THE PURIFICATION OF LACTATE DEHYDROGENASE ISOZYMES LDH-A_4, LDH-B_4 AND LDH-C_4 FROM HUMAN TISSUES

M.R. JISNUSON SVASTI and SUMALEE VIRIYACHAI*

Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok

(*Received 30 January 1975*)

Summary

We have purified all three of the homotetrameric isozymes of human lactate dehydrogenase in milligram quantities. LDH-A_4 was purified 970-fold from human liver to a specific activity of 283 I.U./mg with a yield of 26.5%; LDH-B_4 was purified 60-fold from human heart to a specific activity of 168 I.U./mg with a yield of 72.5%; LDH-C_4 was purified 586-fold from human testes to a specific activity of 80 I.U./mg with a yield of 22%. On polyacrylamide gel electrophoresis, LDH-A_4 and LDH-B_4 were shown to be homogeneous with respect to enzyme activity and to protein. The minor contaminants present in the LDH-C_4 preparation amount to less than 5% of the total protein and less than 2% of the LDH activity. The relative merits and drawbacks of the procedures used are discussed.

Introduction

The enzyme L-lactate dehydrogenase (E.C.1.1.1.27) catalyses the interconversion of pyruvate and lactate[1]. In vertebrates, there are five major isozymic forms LDH-A_4, LDH-A_3B, LDH-A_2B_2, LDH-AB_3 and LDH-B_4, formed by the random combination of two types of polypeptide chains A and B into tetramers. The relative proportions of these five isozymic forms varies considerably from tissue to tissue, with the LDH-A_4 isozyme predominating in skeletal muscle and in liver, and the LDH-B_4 isozyme predominating in cardiac muscle. On the basis of the differences in their kinetic properties, the biological role of the LDH-A_4 isozyme has been proposed to be the conversion of pyruvate to lactate as the terminal reaction of anaerobic glycolysis, while the LDH-B_4 isozyme is thought to be involved in the production of pyruvate from lactate in actively respiring tissues such as heart[2].

More recently a sixth isozyme, LDH-C_4 has been shown to occur only in the mature testes and spermatozoa of vertbrates[3]. The mouse LDH-C_4 isozyme has been shown to differ from the LDH-A_4 and LDH-B_4 isozymes in its kinetic properties, immunological properties and amino acid composition[4,5,6]. The presence of a unique isozyme specific to the male reproductive system suggests that LDH-C_4 may have some special

*Present address: Department of Chemistry, Faculty of Science, Prince of Songkhla University, Haadyai

properties essential for the normal function of spermatozoa. Perhaps the most noteworthy characteristic of the LDH-C_4 isozyme is its ability to utilize a wider range of substrates, such as α-ketobutyrate, than the other isozymes. Because of the species-specificity of the reproductive process, comparative studies of the purified human LDH-A_4, LDH-B_4 and LDH-C_4 are required to clarify the biological role of LDH-C_4 in humans.

The LDH-A_4 and LDH-B_4 isozymes have been purified from many species[1] including humans[7,8,9,10] while the LDH-C_4 isozyme has been purified from mice[5,6] rates[11] and bulls[12], but not from humans. In this paper, the purification of all three of the homotetrameric isozymes LDH-A_4, LDH-B_4 and LDH-C_4 is presented for the first time. The procedures used to isolate human LDH-A_4 and LDH-B_4 are somewhat simpler than those described by other workers. We have previously presented preliminary data cn the purification of human LDH-C_4 [13].

Materials and Methods

Human tissues (liver, heart, and testes) were obtained by autopsy from accident victims with the kind co-operation of the Police Hospital, Bangkok. Specimens were taken within 24 hours affer after death and stored frozen at -20°C until use. Chemicals used were generally of analytical grade.

Enzyme assays: Lactate dehydrogenase activity was measured by following the rate of oxidation of NADH by pyruvate at room temperature (about 25° C), using a Gilford Model 2000 recording spectrophotometer to monitor decreases in optical density at 340 nm. Reactions were carried out in a cuvette of 1 cm light path containing 1 ml of 50 mM potassium phosphate buffer, pH 7.5, 0.30 mM sodium pyruvate and 0.15 mM NADH[6]. The molar extinction coeffient[14] for NADH (6.22×10^6 cm^2 $mole^{-1}$) was used to convert the rate of change of optical density into µmoles NADH utilized in one minute. One international unit (I.U.) of lactate dehydrogenase is defined as the amount of enzyme that will catalyze the oxidation of one µmole of NADH per minute.

Protein determination: The protein concentrations of samples during purification were determined by measuring the optical density at 280 nm and 260 nm uring a Zeiss PNQ spectrophotometer. From the radio of absorbanc at 280 nm to the absorbanc at 260 nm, an appropriate factor was selected to account for nucleic acid contamination. The protein concentration (mg/ml) is the product of the optical dedsity at 280 nm and this factor[15]. But for the purified isozymes, protein content was determined by the method of Lowry[16], using bovine serum albumin as a standard.

Polyacrylamide gel electrophoresis: Electrophoresis was cariied out at 4° C in 0.4 cm x 10 em cylindrical gels containing 5.25% (w/v) polyacrylamide in 10 mM glycine-NaOH buffer, pH 9.8. Samples were run towards the anode for about 2 hours at 200 volts with a current of about 0.6–1.0 mA per tube. Gels were stained for lactate dehydrogenase activity by incubating in the dark for three hours in 10 ml of 0.1 M Tris-HCl, pH 8.0, 0.15 mM NAD^+,

5 mM nitroblue tetrazolium. Other gels were stained for protein with 0.1% amido black in acetic acid-ethanol-water (7:20:73 by volume). In both cases, gels were destained by diffusion into 5% (v/v) acetic acid. The relative proportions of the different isozymes present in any given sample was determined by scanning the destained gels at 600 nm in a Gilford Model 2000 spectrophotometer. The absolute amount of any given isozyme (as reported in Tables I, II and III) is then the product of the total LDH activity and the per cent of that isozyme present as determined by scanning.

Experimental and Results

All purification steps were carried out at 0°–4°C and all solutions were made up with double distilled water. All of the phosphate buffers used contained 1 mM β-mercaptoethanol and 1 mM EDTA.

LDH-A_4 purification: 360 g of human liver was homogenized in 400 ml of ice-cold potassium phosphate buffer pH 7.0. The homogenate was filtered through four layers of cheesecloth and centrifuged at 12,000 g for 40 min. The supernatant was filtered through glass

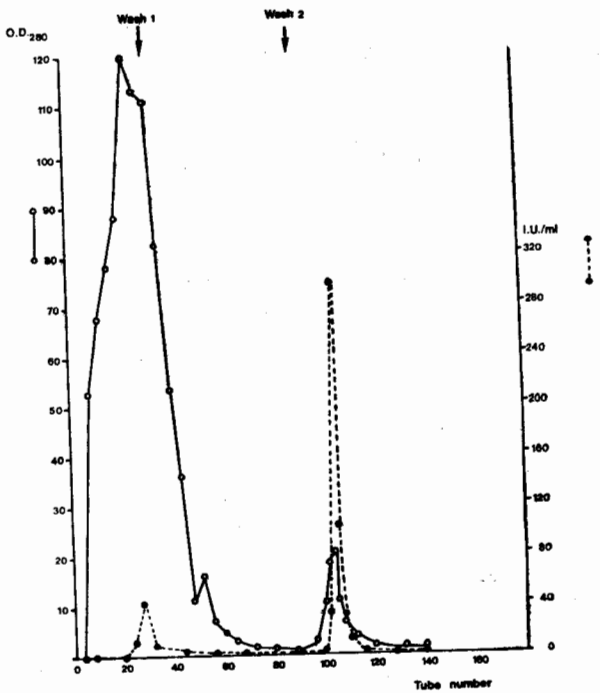

Fig. 1 *Blue dextran Sepharose chromatography of LDH-A_4.* Liver crude extract was applied to a 4 cm x 23 cm column of blue-dextran Sepharose, equilibrated with 10 mM potassium phosphate buffer, pH 7.0. The column was eluted with the same buffer (Wash 1), followed by 0.3 M KCl in the same buffer (Wash 2). Fractions of 17.5 ml per tube were collected. The O.D.280 (0————0) and total LDH activity (■- - - -■) were measured. Tubes 101 to 116 were pooled, dialysed and fractionated by CM-Sephadex chromatography.

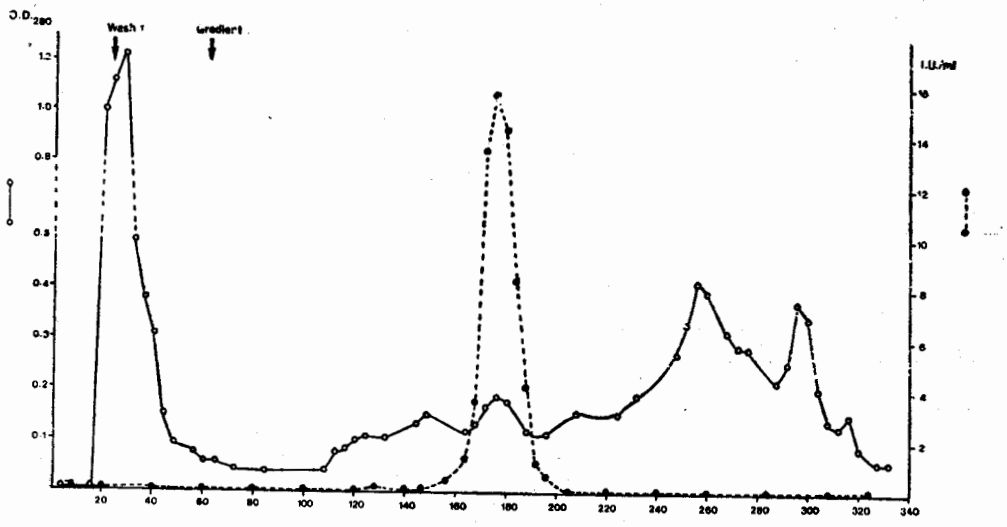

Fig. 2 *CM-Sephadex chromatography of LDH-A_1.* The dialysed blue dextran Sepharose pool was applied to a 5 cm x 40 cm column of CM-Sephadex C–50, equilibrated with 40 mM potassium phosphate, pH 6.0. The column was successively eluted with starting buffer (Wash 1), a linear gradient (2 l + 2 l) of 40 mM potassium phosphate buffer pH 6.0 to pH 9.1 (Gradient), followed by 40 mM potassium phosphate, pH 9.1 (Wash 2). Fractions of 16.5 ml were collected. The O.D.280 (0————0) and total LDH activity (▪– – – –▪) were measured. Tubes 170–186 were pooled and further fractionated.

wool to remove fat. The filtrate (liver crude extract) was applied to a 4 cm x 23 cm column of blue dextran covalently bound to Sepharose 4B (prepared as described by Ryan *et al.*[17]), equilibrated with the same buffer. Most of the proteins could be washed out with starting buffer. Most of the lactate dehydrogenase activity was retained by the column and could be eluted out with 0.3 M KCl in 10 mM potassium phosphate buffer, pH 7.0 (Fig. 1). Tubes 101 to 116 were pooled and dialysed against 40 mM potassium phosphate buffer, pH 6.0.

The dialysed blue dextran pool was applied to a 5 cm x 40 cm column of CM-Sephadex C-50 equilibrated with 40 mM potassium phosphate buffer, pH 6.0 and washed with the same buffer. LDH-A_4 was retained by the column and was eluted out with a linear gradient (2 l + 2 l) of 40 mM potassium phosphate, pH 6.0 to 9.1 (Fig. 2). Tubes 170–186 were pooled and concentrated by dialysis against saturated ammonium sulphate. The precipitate thus obtained was collected by centrifugation at 25,000 g for 30 min and then resuspended in 0.1 M potassium phosphate buffer, pH 7.5 to give a protein concentration of 5 mg/ml. Solid ammonium sulphate was added to give 40% saturation. The supernatant was then repeatedly fractionated with solid ammonium sulphate to give 5% increments in saturation until 65% saturation was reached. The bulk of the enzyme activity was found in the precipitates at 55% and 60% saturation, which were therefore pooled. This purification procedure is summarized in Table I.

Table I

Summary of human LDH-A_4 purification

Sample	Total LDH-A_4 activity (I.U.)	Total protein (mg)	Specific activity (I.U. LDH-A_4/mg)	Purification	% Yield
Liver crude extract	13,600	45,000	0.29	1	100
Blue dextran Sepharose pool	8,600	2,080	4.14	14.3	64
Dialysed blue dextran pool	6,900	1,540	4.46	15.1	51
CM-Sephadex pool	4,000	72.5	55.0	190	29.5
50–60% $(NH_4)_2SO_4$ ppte	3,600	12.7	283	970	26.5

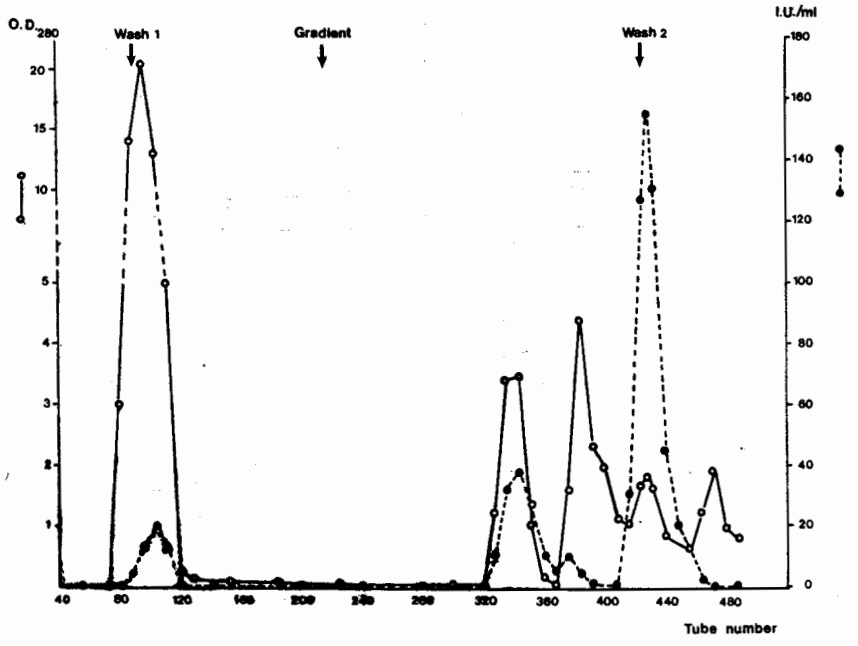

Fig. 3 *DEAE-Cellulose chromatography of LDH-B_4.* The dialysed 25%–60% ammonium sulphate cut was applied to a 5 cm x 50 cm column of DEAE-cellulose equilibrated with 5 mM potassium phosphate pH 7.0 The column was successively eluted with starting buffer (Wash 1), a linear gradient (500 ml + 500 ml) of 0 to 0.3 M NaCl in the same buffer (Gradient), followed by 0.3 M NaCl in the same buffer (Wash 2). The O.D.280 (0————0) and total LDH activity (■- - -■) were measured. Fractions of 4.8 ml were collected, and tubes 416 to 442 were pooled.

LDH-B_4 purification: 700 g of human hearts were homogenized in 950 ml of 1 mM EDTA–1 mM B-mercaptoethanol The homogenate was filtered through cheesecloth and centrifuged at 12,000 g for one hour. The supernatant was filtered through glass wool and the filtrate (heart crude extract) was made up to be 70% saturated in ammonium sulphate. The precipitate was collected by centrifugation at 12,000 g for one hour and made up to 25% saturation in ammonium sulphate by the addition of 1 mM EDTA-1 mM β-mercaptoethanol. This suspension was centrifuged at 12,000 g for one hour and the resulting supernatant made up to 60% saturation by addition of solid ammonium sulphate. The precipitate was collected by centrifugation at 12,000 g, dissolved in 40 ml of 1 mM EDTA-1 mM β-mercaptoethanol and dialysed against 5 mM potassium phosphate buffer, pH 7.0. The dialysate was applied to a 5 cm x 50 cm column of DEAE-cellulose (Cellex-D) equilibrated with the same buffer. LDH-B_4 is the isozyme most strongly adsorbed by this column and could be eluted out free of other isozymes with a linear gradient (500 ml + 500 ml) of 0 to 0.3 M NaCl in 5 mM potassium phosphate buffer, pH 7.0 (Fig. 3). Tubes 416 to 442 were pooled and precipitated by adding solid ammonium sulphate to 70% saturation. This purification procedure is summarized in Table II.

Table II

Summary of human LDH-B_4 purification

Sample	Total LDH-B_4 activity (I.U)	Total protein (mg)	Specific activity (I.U. LDH-B_4/mg)	Purification	% Yield
Heart crude extract	11,100	3,920	2.83	1	100
25–60% $(NH_4)_2SO_4$	12,300	3,760	3.28	1.2	(110)
Dialysed $(NH_4)_2SO_4$ Cut	9,200	2,340	3.93	1.4	84
DEAE-Cellulose pool	8,000	47.5	168	59.5	72.5

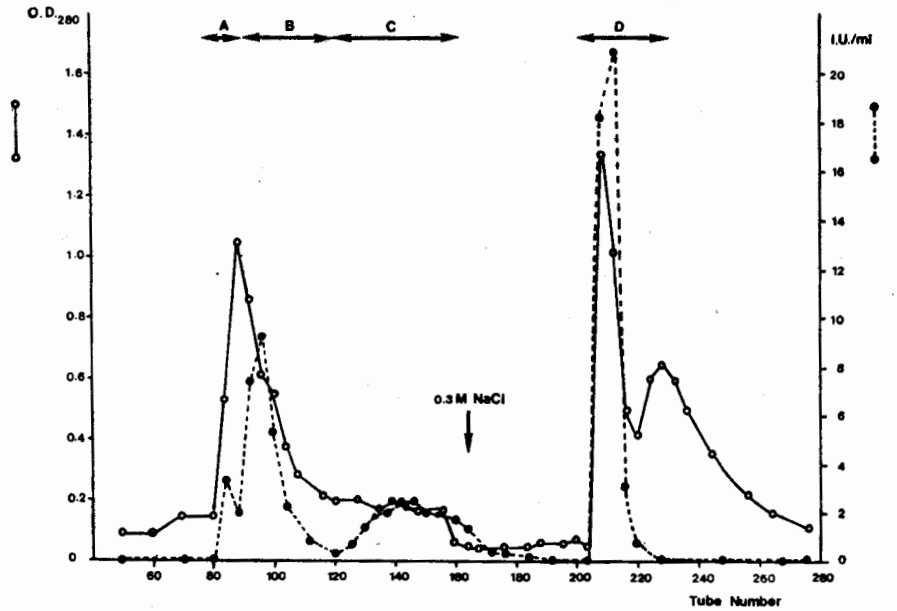

Fig. 4 *Second DEAE-Cellulose chromatography of LDH-C_4.* The dialysed breakthrough peak from the first DEAE-cellulose column was applied to a second DEAE-cellulose column (4.5 cm x 60 cm), equilibrated with 10 mM potassium phosphate buffer, pH 7.5 The column was eluted with 1.5 l of starting buffer, followed by 0.3 M NaCl in 10 mM potassium phosphate buffer, pH 7.5 For tubes 0 to 164 fractions of 6 ml were clolected and for tubes 165 to 278 fractions of 12 ml were collected. O.D.280 (0———0) and total LDH activity (▪- - - -▪) were measured. Four pools (A, B, C and D) were taken.

LDH-C_4 purification: 225 gm of human testes were homogenized in 250 ml of 1 mM EDTA-1 mM β-mercaptoethanol. The homogenate was filtered through cheesecloth and centrifuged at 12,000 g for one hour. The supernatant (testis crude extract) was fractionated by addition of solid ammonium sulphate to give 70% saturation. The precipitate was collected by centrifugation, resuspended in 10 mM potassium phosphate, pH 7.5 to give a protein concentration of about 30 mg/ml and fractionated with ammonium sulphate. The fraction precipitating at between 40% and 60% saturation was dissolved in 10 mM potassium phosphate buffer, pH 7.5 and dialysed against the same buffer.

The dialysed sample was loaded on to a 4.5 cm x 50 cm column of DEAE-cellulose equilibrated with the same buffer. Since very little LDH activity was retained by this column, the breakthrough peak was precipitated with ammonium sulphate at 70% saturation, dialysed against 10 mM potassium phosphate buffer, pH 7.5 and applied to another DEAE-cellulose column (4.5 cm x 60 cm) equilibrated with the same buffer. This column was washed with 1.5 litres of starting buffer, followed by 2 litres of 0.3 M NaCl in 10 mM potassium phosphate, pH 7.5 (Fig. 4). Four pools (A, B, C and C) were precipitated with ammonium sulphate to 70% saturation and small samples of each pool were subjected to electrophoretic analysis (Fig. 5). LDH-C_4 was present in both pools C and D. In pool D,

Table III

Summary of human LDH-C_4 purification

Sample	Total LDH-C_4 activity (I.U.)	Total protein (mg)	Specific activity (I.U. LDH-C_4/mg)	Purification	% Yield
Testis crude extract	1,520	10,580	0.14	1	100
70% $(NH_4)_2SO_4$ ppte	1,520	3,740	0.40	2.86	100
40–60% $(NH_4)_2SO_4$ ppte	1,100	2,125	0.51	3.64	72
First DEAE-Cellulose	1,035	840	1.23	8.80	68
Second DEAE-Cellulose (Pool C)	500	18	27.8	199	33
70% $(NH_4)_2SO_4$ ppte (Pool C)	335	4.1	82.0	586	22

it only comprised less than 10% of the total LDH activity, while in pool C, it appeared to be the only isozyme present. The identification of pool C as LDH-C_4 was confirmed by its ability to utilize α-ketobutyrate as a substrate far better than either the LDH-A_4 or LDH-B_4 isozymes can[13]. This purification procedure is summarized in Table III.

Gel electrophoresis of purified isozymes: Duplicate samples of each of the purified isozymes were subjected to polyacrylamide gel electrophoresis. One gel was stained for lactate dehydrogenase activity and the other gel for protein and the results are shown in Fig. 6. No other proteins or isozymes are present in the gels of LDH-A_4 and LDH-B_4 (Fig. 6a, 6b), indicating that these preparations are of high purity. Some minor contaminants are present in the gels of LDH-C_4 but comprise less than 5% of the total protein and less than 2% of the total LDH activity.

Discussion

Nisselbaum *et al.* have described the isolation of LDH-A_4 from human liver[8] and LDH-B_4 from human heart[7] using rather laborious purification procedures resulting in relatively low yields (14% for LDH-A_4 and 13% for LDH-B_4). More recently, Burd *et al.*[10] have described a less cumbersome general scheme for the isolation of human LDH-A_4 from liver and human LDH-B_4 from erythrocytes involving the use of calcium phosphate gel absorption, ammonium sulphate fractionation, DEAE-Sephadex and Sephadex G-100 chromatography. They obtained yields of 23% for LDH-A_4 and 10% for LDH-B_4. The procedures used in the present study to isolate LDH-A_4 and LDH-B_4 are much less complex. It is difficult to compare the specific activities of our preparations with those of previous studies because different workers express their results in different units. But from the polyacrylamide gels shown in Fig. 6, our preparations of LDH-A_4 and LDH-B_4 are unlikely to be any less pure than others previously described.

Human LDH-B_4 was by far the simplest of the isozymes to purify, partly because of its high initial specific activity compared to those of LDH-A_4 and LDH-C_4 and partly because of its low isoelectric point. Our procedure for isolating LDH-B_4 (Table II) was modified from that of Pesce *et al.*[9] and differs from it only in the omission of acetone fractionation. In our experience, acetone fractionation causes a considerable loss of LDH activity and is not necessary since the subsequent DEAE-cellulose chromatography provides sufficient purification. By making this omission, we were able to isolate LDH-B_4 with more than double the yield obtained by Pesce *et al.*[9] (33%). Although our yield of human LDH-A_4 (Table I) is no better than that of Burd *et al.*[10], we were able to avoid the use of so many purification steps by having, as the first step, chromatography on blue dextran covalently attached to Sepharose[17]. This single step gave a 14-fold purification, which made it possible to use CM-Sephadex chromatography as the next step (after dialysis), without any danger of overloading the column. The yield of the affinity chromatography step was lower than expected but it should be possible to improve this by using a larger amount of blue dextran Sepharose.

Wong *et al.*[6] and Goldberg[5] have independently used the greater heat stability and higher isoelectric point of mouse LDH-C_4 to purify it free of other isozymes to a speci-

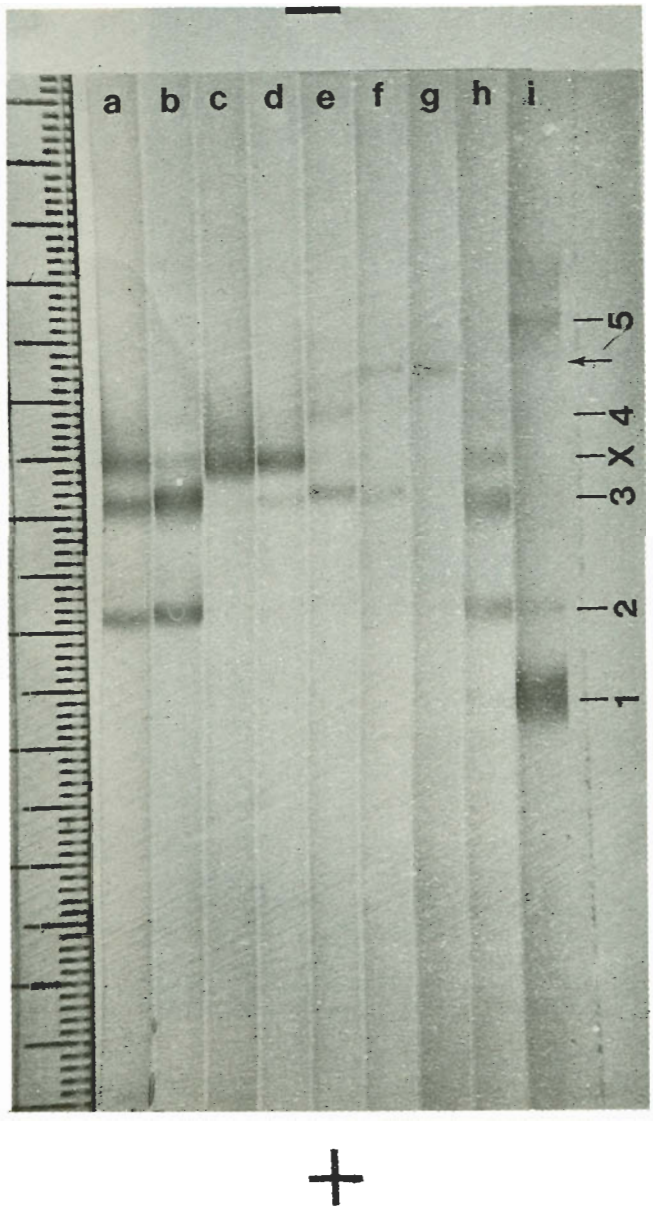

Fig. 5 *Polyacrylamide gel electrophoresis of DEAE-cellulose pools.* Samples of the DEAE-cellulose pools A, B, C and D (Fig. 4) were run on 5.25% polyacrylamide gels in 10 mM glycine-NaOH buffer pH 9.8 and stained for LDH activity. *Gel a:* pool C + pool D; *gel b:* pool D; *gel c:* pool C; *gel d:* pool B + pool C; *gel e:* pool B; *gel f:* pool A + pool B; *gel g:* pool A; *gel h:* testis crude extract; *gel i:* mixture of partially purified LDH-A_4 and LDH-B_4. Markings at the side 1, 2, 3, 4, 5 and X indicate the bands corresponding to LDH isozymes B_4, AB_3, A_2B_2, A_3B, A_4 and C_4 respectively. Arrow indicates the presence of a band (in gels f and g) not present in the crude extract.

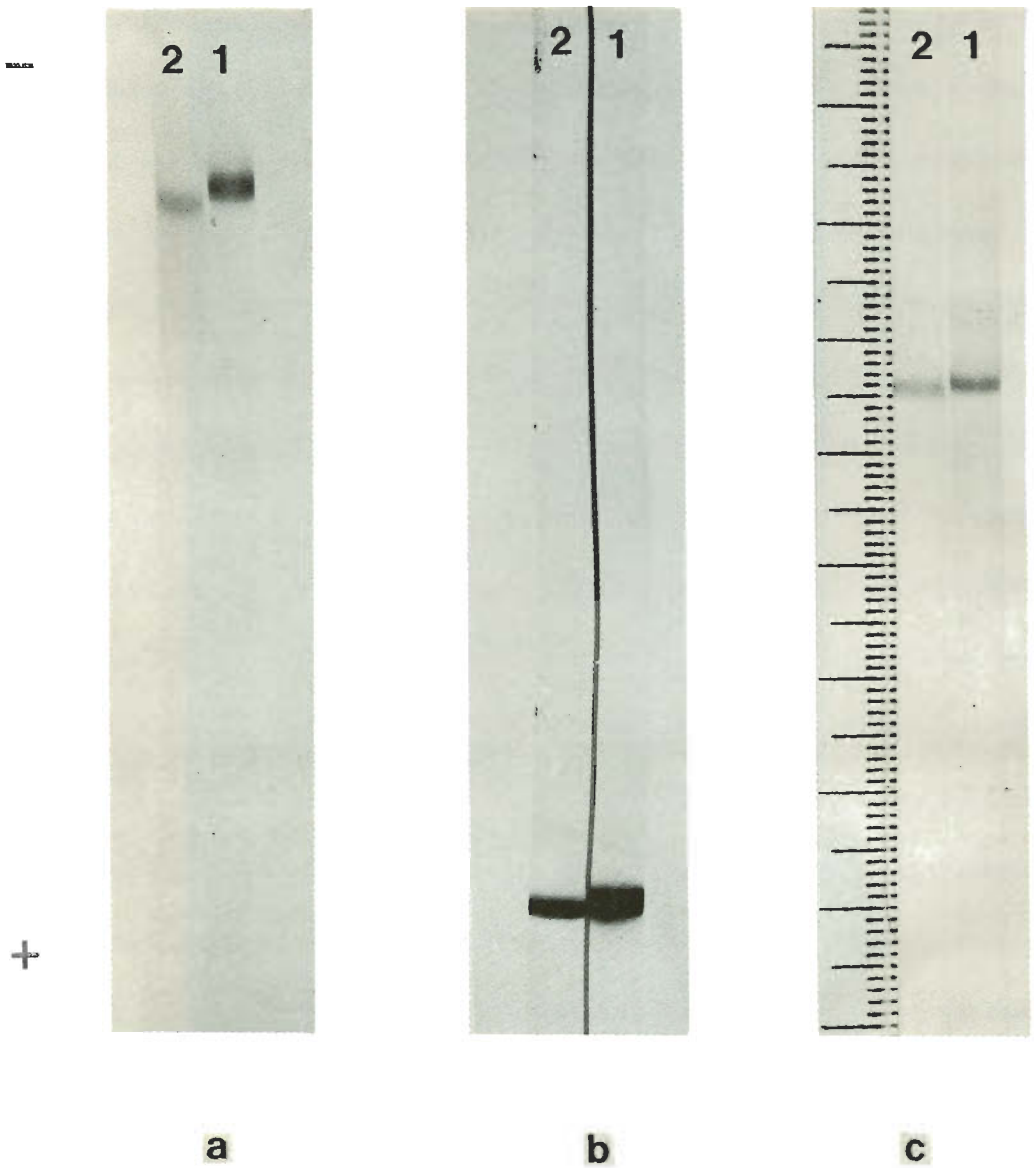

Fig. 6 *Polyacrylamide gel electrophoresis of LDH-A_4.* Duplicate samples of the purified isozymes were run on 5.35% polyacrylamide gels in 10 mM glycine-NaOH buffer, pH 9.8 at 200 volts for about 2 hours. One sample (1) was stained for LDH activity and the other (2) was stained for protein. (a) LDH-A_4; (b) LDH-B_4; (c) LDH-C_4.

fic activity of about 32 I.U./mg. Unfortunately, human LDH-C_4 is not much more heat stable than other human isozymes and has an electrophoretic mobility in between LDH-A_2B_2 and LDH-A_3B. Bovine LDH-C_4, which has similar disadvantages to human LDH-C_4, has been purified to homogeneity but only 3 mg enzyme was obtained from 3 Kg of bull testes[12]. In our purification of LDH-C_4, although some very minor contaminants are still present, 4 mg of LDH-C_4 were obtained with a specific activity of 80 I.U./mg from only 225 gm of human testes.

There are three interesting points concerning our purification procedure for human LDH-C_4 (Table III). Firstly, the large increase in specific activity in the last step is a little surprising as ammonium sulphate precipitation has already been used in previous steps. This observation may be due to the fact that the protein concentration in pool C (0.33 mg/ml) was very low so that not all the proteins were precipitated. Secondly, in the gels shown in Fig. 5, an extra band (marked by arrow), with too low a yield to be observed in testis crude extract, is present between LDH-A_3B and LDH-A_4. One possible explanation is that in humans there are multiple C loci as is sometimes found in pigeons[18]. A more likely explanation is that this band may represent a hybrid form between the A subunit and the C subunit as was found in the rat and the guinea pig[19]. But in our preparation this may not be an *in vivo* phenomenon because testes were stored frozen on collection and thawed before use, so that hybridization might have occurred as an artifact.

Finally, in the first DEAE-cellulose column LDH-C_4 was eluted out in the void volume producing a two-fold increase in specific activity but in the second DEAE-cellulose column LDH-C_4 is retarded and can be eluted out with starting buffer in pool C with a 23-fold purification. These different results could be due to slight unintentional differences in the chromatographic conditions used, such as pH or concentrations of buffer. Alternatively, they may be caused by the differences in amount of total protein loaded (2,125 mg in the first column and 840 mg in the second column). Such minor differences may be of importance since the present conditions appear to be close to the limit at which LDH-C_4 can be retarded. Similar explanations may account for the differences between our results and those of Blanco *et al.*[20], who found that human LDH-C_4 is retained by DEAE-cellulose in the presence of 10 mM sodium phosphate buffer, pH 7.0 and could be eluted out, contaminated with LDH-A_2B_2, with 75 mM NaCl. Nevertheless, we have been able to obain reproducible separation of human LDH-C_4 from other human LDH isozymes using DEAE-cellulose chromatography with a low loading of total protein. But the specific activity of LDH-C_4 obtained is not reproducibly high, so that a further fractionation step using affinity chromatography on NAD^+–Sepharose[21] or oxamate-Sepharose[22] may be required. The least satisfactory aspect of separating LDH-C_4 from other isozymes on DEAE-cellulose is the fact that some LDH-C_4 is also found in pool B and in pool D (Fig. 5), making the yield of LDH-C_4 in pool C rather low (Table III). We have explored other possible methods for separating LDH-C_4 from other isozymes, such as CM-Sephadex chromatography and preparative isoelectric focussing, but the results have so far not been satisfactory.

Nomenclature

The nomenclature for LDH isozymes used in this paper is the one recently adopted at the Third International Conference on Isozymes, held at Yale University on

April 17–20, 1974. Alternative names for LDH-A_4 and LDH-B_4 are M_4, LDH-1, LHD-5 and H_4, LDH-5, LDH-1 respectively. LDH-C_4 is also known as LDH-X.

Acknowledgements

We thank the Faculty of Science, Mahidol University, and the Rockefeller Foundation for providing research support. Some of this work was carried out as part of S.V.'s M.Sc. dissertation, during which time she received a research studentship from the Prince of Songkhla University. We would also like to thank Ms. Panor Thongtem for helping with the LDH-B_4 purification and Ms. Thitika Vatcharotai for assistance in the preparation of this manuscript.

References

1. Everse, J. and Kaplan, N.O. (1973) in *Advances in Enzymology* (Meister, A., ed.), vol. 37, pp 61–133, Academic Press, New York.
2. Dawson, D.M., Goodfriend, T.L. and Kaplan, N.O. (1964) *Science* **143,** 923–933.
3. Zinkham, W.H., Blanco, A., Clowry. L.J. (1964) *Ann. N.Y. Acad. Sci.* **121,** 571–588.
4. Goldberg, E. (1971) *Proc. Nat. Acad. Sci.* **68,** 349–352.
5. Goldberg, E. (1972) *J. Biol. Chem.* **247,** 2044-2048.
6. Wong, C., Yanez, R., Brown, D.M., Dickey, A., Parks, M.E. and Mc Kee, R.W. (1971) *Arch. Biochem. Biophys.* **146,** 454–460.
7. Nisselbaum, J.S. and Bodansky, O. (1961) *J. Biol. Chem.* **236,** 323–330.
8. Nisselbaum, J.S. and Bodansky, O. (1963) *J. Biol. Chem.* **238,** 969–974.
9. Pesce, A., Mc Kay, R.H., Stolzenbach, F., Castillo, F. and Kaplan, N.O. (1967) *J. Biol. Chem.* **242,** 2151–2167.
10. Burd, J.F. and Usategui-Gomez, M. (1973) *Biochim. Biophys. Acta* **310,** 238–247.
11. Schatz, L. and Segal, H.L. (1969) *J. Biol. Chem.* **244,** 4393–4397.
12. Kolb, E., Fleisher, G.A. and Larner, J. (1970) *Biochemistry* **9,** 4372–4380.
13. Svasti, J. and Viriyachai, S. (1974) in *Proceedings of the Third International Conference on Isozymes* (Markert, C.L., ed.), vol. 2 pp 113–127, Academic Press, New York.
14. Horecker, B.L. and Kornberg, A. (1948) *J. Biol. Chem.* **175,** 385.
15. Chaykin, S. (1966) *Biochemistry Laboratory Techniques,* p. 137, John Wiley and Sons Inc., New York.
16. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) *J. Biol. Chem.* **193,** 265-275.
17. Ryan, L.D. and Vestling, C.S. (1974) *Arch. Biochem. Biophys.* **160,** 279–284.
18. Zinkham, W.H., Blanco, A. and Kupchyk, R.L. (1964) *Science* **144,** 1353–1354.
19. Goldberg, E. (1973) *J. Exp. Zool.* **186,** 273–278.

20. Blanco, A. and Zinkham, W.H. (1963) *Science* **139**, 601–603.
21. Mosbach, H., Guilford, H., Ohlsson, R. and Scott, M. (1972) *Biochem. J.* **127**, 625–631.
22. O'Carra, P. and Barry, S. (1972) *FEBS Letters* **21**, 281–285.

บทคัดย่อ

เราได้แยก homotetrameric isozymes ของ L–Lactate dehydrogenase (LDH) ทั้ง ๓ ชนิดจากเนื้อเยื่อของคน และทำให้บริสุทธิ์ได้ปริมาณเป็นมิลลิกรัม LDH – A_4 จากตับคนสามารถทำให้บริสุทธิ์ขึ้น 970 เท่าและมี specific activity 283 I.U./mg LDH–B_4 จากหัวใจคนทำให้บริสุทธิ์ขึ้นเป็น 60 เท่า และมี specific activity 168 I.U./mg ส่วน LDH – C_4 จากอัณฑะคนทำให้บริสุทธิ์ขึ้นได้ 586 เท่า และมี specific activity 80 I.U./mg ปริมาณ LDH ทั้งสามชนิดที่ได้รับจากการทำให้บริสุทธิ์แล้ว คิดเปรียบเทียบกับปริมาณที่มีอยู่เดิมทั้งหมดในเนื้อเยื่อเป็น 26.5 %, 72.5 % และ 22 % ตามลำดับ การวิเคราะห์โดยใช้ polyacrylamide gel electrophoresis ได้ผลว่า LDH – A_4 และ LDH – B_4 ที่ได้นั้น ต่างประกอบด้วยโปรตีนชนิดเดียว และมี activity ของเอ็นไซม์เดียวเท่านั้น ส่วน LDH – C_4 ยังมีโปรตีนอื่นปนอยู่ด้วย โปรตีนที่ปนอยู่นี้คิดเป็น 5 % และมี activity ของเอ็นไซม์ราว 2 % ของ LDH – C_4 ที่ได้ เราได้วิจารณ์ถึงข้อดีและข้อเสียของวิธีดำเนินการทดลองที่ใช้ไว้ในบทความนี้ด้วย