Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 5260–5264, August 1985
Biochemistry

Identification of a nucleic acid helix-destabilizing protein from rat liver as lactate dehydrogenase-5

(single-stranded DNA binding protein/chemical modification/protein sequences)

KENNETH R. WILLIAMS*, SESHA REDDIGARI†, AND GORDHAN L. PATEL†‡

†Department of Zoology, University of Georgia, Athens, GA 30602; and *Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06510

Communicated by Norman H. Giles, April 8, 1985

ABSTRACT **A rat liver DNA helix-destabilizing protein (HDP) that has previously been proposed to play a role in transcription has been identified as M chain lactate dehydrogenase (LDH-5; L-lactate:NAD$^+$ oxidoreductase, EC 1.1.1.27). Tryptic peptides accounting for 157 amino acids in the rat liver HDP have been characterized and then matched to the published sequence for the M chain of porcine LDH. Based on amino acid compositions and direct solid-phase protein sequencing, at least 148 of the 157 residues that were compared are identical in both proteins. In addition, both porcine LDH and the rat liver HDP have blocked amino termini and similar amino acid compositions and molecular weights. Rat liver HDP and LDH-5 that were purified to molecular homogeneity had similar specific activities in both single-stranded DNA (ss DNA) binding and LDH assays. HPLC tryptic peptide maps were also identical for both the rat liver HDP and LDH proteins. Since preincubation of HDP in NADH prevents its binding to ss DNA, both NADH and ss DNA may be binding at the same site. Further support for this latter idea derives from chemical-modification studies which demonstrate that tyrosine-238, which is located near the coenzyme binding site of LDH, seems to be essential for the ability of HDP to bind ss DNA. These results indicate caution in ascribing *in vivo* roles solely on the basis of binding to ss DNA. Alternatively, they suggest that a single protein may play more than one biological role.**

Proteins that bind in a stoichiometric fashion to single-stranded DNA (ss DNA) and have no known enzymatic activity have been isolated from a large variety of prokaryotic and eukaryotic sources (see refs. 1–3 for recent reviews). Because of the availability of suitable mutants, it is now well documented that at least two prokaryotic ss DNA binding proteins, those from bacteriophage T4 (gene *32* protein) and *Escherichia coli* (SSB protein), play essential roles in DNA replication, repair, and recombination *in vivo* (1–3). While functionally homologous proteins might be expected to occur in eukaryotes, it also has been suggested that there may be similar ss DNA and RNA binding proteins that could be envisioned to play equally important roles in DNA transcription, RNA processing, and transport as well as protein synthesis (see ref. 4 for a review). In support of this idea, a 30,000-dalton protein, which binds ss DNA and destabilizes the DNA double helix *in vitro*, has been purified from rat liver (5, 6). Antisera to this protein have been shown to cross-react with a protein that appears to be present in various regions of *Drosophila* salivary gland cells but which is highly concentrated at transcriptionally active puffs of polytene chromosomes (7). Thus, it was proposed that the rat ss DNA binding protein might play a role in some phase of transcription. The present study was undertaken to explore structure–function relationships in this rat liver helix-destabilizing protein (HDP). Aromatic amino acids have been shown to be involved in the binding of several prokaryotic HDPs to ss DNA (8–10). It has been proposed that the presence of a set of sequentially placed aromatic amino acid side chains may confer a regular ladder pattern on the bases in single-stranded polynucleotides and that this mechanism of binding may be common to all single-stranded nucleic acid binding proteins (9). Based on studies on oligopeptides containing aromatic amino acids (see ref. 11 for a review), these proposed "stacking" interactions might account for the ability of this class of proteins to specifically recognize single-stranded as opposed to double-stranded nucleic acids. To determine if aromatic amino acids might also be essential for rat liver HDP binding to ss DNA, we used chemical reagents to specifically modify tyrosine residues in this protein and then a filter binding assay to determine the effect of this modification on the DNA binding activity of this HDP. Our results showed that indeed HDP contains a tyrosine critical in DNA binding. Determination of amino acid sequence of the peptide containing this tyrosine residue and subsequent screening of the Protein Data Base by the National Biomedical Research Foundation led us to the first indication of homology between HDP and lactate dehydrogenase (LDH; L-lactate:NAD$^+$ oxidoreductase, EC 1.1.1.27).

MATERIALS AND METHODS

Purification of Rat Liver HDP and LDH. The rat liver HDP was isolated as described (5, 6) by affinity chromatography on native double-stranded DNA-cellulose and ss DNA-cellulose columns prepared as described by Alberts and Herrick (12). Final purification was achieved by gel filtration of proteins eluted from ss DNA-cellulose on Ultragel AcA 44 (LKB). The rat liver LDH was purified by sequential chromatography of tissue extract on Cibacron blue-Sepharose and DEAE-cellulose, followed by affinity chromatography on N^6-(6-aminohexyl)-AMP-Sepharose (13) provided by Sangram Sisodia in this laboratory.

DNA Binding and LDH assays. ss DNA binding activity was measured by nitrocellulose filter binding (14) and utilized heat-denatured [^{32}P]DNA that had been labeled by nick-translation. Each assay contained 0.1 μg of [^{32}P]DNA (2400 cpm) and 2.5 μg of HDP in a total volume of 1.1 ml of buffer A (5 mM Tris·HCl, pH 8.0/1 mM EDTA/0.1 mM dithiothreitol/10 mM NaCl/10% glycerol). After incubation at room temperature for 15 min, the DNA/protein mixture was filtered through nitrocellulose filter discs. The LDH assay used 0.1 M Tris·HCl, pH 8.0/1 mM Na pyruvate/0.15 mM NADH and was based on that of Lee *et al.* (13).

Chemical Modification of HDP. Nitration of the HDP was done by the method of Sokolovsky *et al.* (15) in 0.05 or 0.1

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ss DNA, single-stranded DNA; HDP, helix-destabilizing or ss DNA binding protein; LDH, lactate dehydrogenase.

‡To whom reprint requests should be addressed.

M Tris·HCl (pH 8.0) at a protein concentration of 0.3 mg/ml or less. The reaction was allowed to proceed for 90 min at room temperature before it was quenched with 2-mercaptoethanol. After dialysis, the extent of nitration was determined by measuring the absorbance at 381 nm assuming an extinction coefficient of 2200 $M^{-1}\cdot cm^{-1}$ (15) and by amino acid analysis. HDP was iodinated with KI_3 by the method described by Fanning (16). $Na^{125}I$ was added to a stock solution of 0.01 M resublimed iodine in 0.1 M KI. Appropriate amounts of this reagent were then added to HDP (0.2–0.5 mg/ml) in 0.05 or 0.1 M sodium borate buffer (pH 9.0) at 0°C. After 60 min, the reaction was stopped by adding 2-mercaptoethanol prior to dialysis.

Protein Chemistry Studies. Tryptic digests were done in 2 M urea/25 mM NH_4HCO_3 at a protein/enzyme ratio of 25:1 (wt/wt). After 24 hr at 37°C, the digest was stopped by injecting it directly onto a Waters C_{18} μBondapak column equilibrated at a flow rate of 0.7 $ml\cdot min^{-1}$ with 10 mM potassium phosphate (pH 2.5). Peptides were eluted with increasing concentrations of acetonitrile as follows: 0–30% (0–86 min), 30–60% (86–129 min), and 60–100% (129–143 min). Those peptides requiring further purification were dried under reduced pressure, redissolved in 1 ml of 2 M urea, and then reinjected onto the same column equilibrated with 10 mM potassium phosphate (pH 6.0). The column was then eluted with acetonitrile gradients as above. Amino acid analyses were performed on a Beckman 121 M analyzer. Peptides selected for sequencing were coupled to aminopolystyrene and then subjected to solid-phase sequencing on a Sequemat Mini-15 sequencer as described (17).

RESULTS

Rat Liver HDP Contains a Tyrosine Residue Essential for ss DNA Binding. Modification with tetranitromethane has been used to demonstrate that the ss DNA binding proteins from bacteriophage fd (18) and T4 (19) contain essential tyrosine residues. A similar result was obtained for the rat liver HDP (Fig. 1). Modification of two of the six tyrosine residues in this protein resulted in >95% loss in ss DNA binding activity as measured by a nitrocellulose filter assay (Fig. 1*A*) or by HDP-induced melting of poly[d(A-T)] (data not shown). Amino acid analyses verified that nitration of 0.54 molecules of tyrosine per HDP subunit had no effect on the tryptophan, histidine, or methionine residues, which suggested that this reaction had been restricted solely to tyrosine. Further evidence supporting this conclusion was from 5,5-dithiobis(2-nitrobenzoic acid) titrations of the native and nitrated HDP and from comparative peptide mapping studies described more extensively below, which indicated that nitration of rat liver HDP only altered the elution position of a single tyrosine-containing tryptic peptide. In addition, $NaDodSO_4$/gel electrophoresis verified that the modified HDP migrated as a 30,000-dalton species, thus ruling out the possibility of tetranitromethane-induced protein–protein crosslinking. Although complete inactivation of the ss DNA binding activity of the rat liver HDP was accompanied by modification of two tyrosine residues, the protection experiment shown in Fig. 1*B* suggests that only one of the modified tyrosines is actually essential for DNA binding. As shown in this figure, ss DNA significantly decreased the rate of reaction of HDP with tetranitromethane. At tetranitromethane-to-HDP-monomer ratios of >100, this protection corresponds to an average of only one tyrosine per HDP monomer. Similar results to those shown in Fig. 1 were also observed upon iodination of HDP (data not shown).

Identification of the Essential Tyrosine Residue in Rat Liver HDP. Comparative tryptic peptide mapping was used to rapidly identify the particular tyrosine residue in the rat liver HDP that is essential for ss DNA binding. In this experiment (Fig. 2), reverse-phase HPLC was used to fractionate tryptic peptides from 28.7 nmol of the native rat liver HDP (Fig. 2 *Top*) or from the same amount of this protein that had been nitrated (Fig. 2 *Middle*) or iodinated (Fig. 2 *Bottom*) to such an extent that 50% of the DNA binding activity had been lost. Amino acid analyses of all major peaks from the control digest indicated that at least 13 peaks corresponded to relatively pure tryptic peptides and that 4 of these (labeled T-3, T-7, T-10, and T-12 in Fig. 2) contained a single tyrosine residue each. The only major alteration that was detected after nitration was the appearance of a new peak at about 72 min that corresponded by amino acid analysis to the nitrated T-12. The yield of the nitrated T-12 was 3.9 nmol compared to 14.3 nmol of native T-12. These values can be compared with a yield of 16.9 nmol of T-12 from the control tryptic digest. Based on the relative recoveries of the nitrated and nonnitrated T-12, it would appear that 50% inactivation of the rat liver HDP can be achieved by tetranitromethane modification of only about 21% of the rat liver HDP monomers—a finding that suggests that inactivation does not require that all monomers in the HDP tetramer be modified. Extensive amino acid analyses confirmed that there was no decrease in the yield of any other tyrosine-containing tryptic peptide nor was there any nitrotyrosine detected in any other peak obtained from the chromatogram.

Iodination decreased the yield of T-12 to <4.5 nmol and resulted in the appearance of two major ^{125}I-labeled peaks

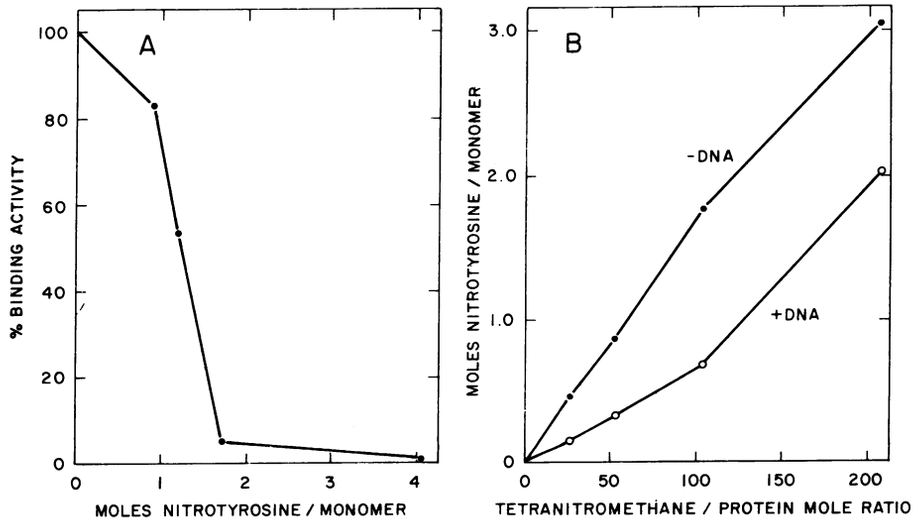

FIG. 1. (*A*) Effect of nitration on ss DNA binding by rat liver HDP. Heat-denatured [^{32}P]DNA (0.1 μg) was incubated at room temperature for 30 min with 2.5 μg of HDP that had been previously modified with various molar excesses of tetranitromethane. The amount of [^{32}P]DNA complexed by these HDP samples was determined by a nitrocellulose filter binding assay, in which the amount of DNA retained by a control was taken to represent 100% activity. Each point was an average of three separate incubations. (*B*) Nitration of HDP in the presence and absence of ss DNA. Aliquots of HDP (11.5 nmol) were nitrated with 26, 52, 104, and 208 molar excess of tetranitromethane either in the presence or absence of heat-denatured calf thymus DNA. The ratio of protein to DNA was 15:1 (wt/wt).

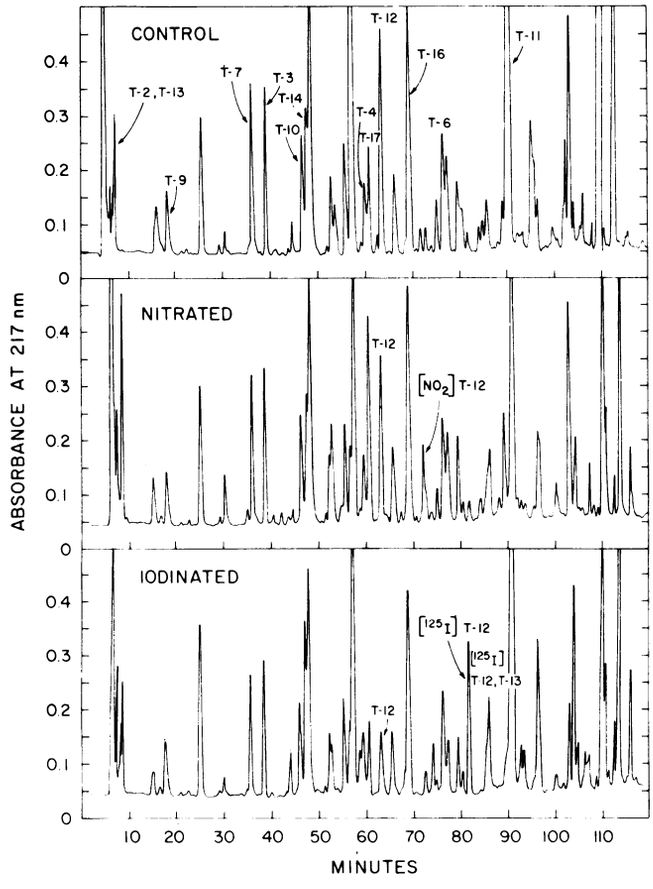

FIG. 2. HPLC separation of tryptic peptides from 28.7 nmol of the native rat liver HDP (*Top*) or from the same amount of this protein that had been nitrated (*Middle*) or iodinated (*Bottom*) to such an extent that 50% of the DNA binding activity had been lost. The Waters C_{18} column was eluted with acetonitrile gradients as described.

that were eluted at about 81 and 85 min, respectively (Fig. 2 *Bottom*). Amino acid analysis indicated that the peak that was eluted at 81 min corresponds to iodinated T-12, and the peak fraction eluted at 85 min has a composition identical to that of T-12 except that, in addition, this fraction contains one extra leucine and lysine residue. This latter composition exactly matches that expected for T-12 and T-13 (see Fig. 3 below), which suggests that iodination has decreased the rate of tryptic cleavage after T-12, resulting in the appearance of the overlapping peptide containing both T-12 and T-13. To ensure that no other peptides had been iodinated, the entire gradient shown at the bottom of Fig. 2 was collected in 1.5-ml Eppendorf tubes and subjected to gamma radiation assay. The iodinated T-12 and T-12,13 accounted for more than 44% and 17%, respectively, of the recovered cpm. The remaining radioactivity was found in numerous small peaks, none of which contained >8% of the total radioactivity recovered. Both the native and iodinated T-12 were subjected to solid-phase sequencing, which resulted in the following sequence: Glu-Val-Val-Asp-Ser-Ala-Tyr-Glu-Val-Ile-Lys, which exactly agreed with the amino acid composition that was determined for this peptide (data not shown).

Identification of the Rat Liver HDP as LDH. In order to determine if other ss DNA binding proteins might contain a similar active site tyrosine, we submitted the sequence of T-12 to the National Biomedical Research Foundation. Their computerized search of the Protein Data Base revealed that porcine M chain LDH contained a sequence spanning residues 232–242 that was identical to the sequence that we determined for T-12 isolated from rat liver HDP. The homology between these two proteins was further confirmed (Fig. 3) by direct sequencing of 10 rat liver HDP tryptic peptides and by matching the amino acid compositions of an additional 4 HDP tryptic peptides to the known sequence of the porcine LDH M chain (20). Together these peptides account for 157 residues in HDP, and our analysis indicates that at least 148 of these residues are identical in porcine LDH M chain and our rat liver HDP. In addition, both proteins have a blocked amino terminus and similar amino acid compositions and form stable 120,000-dalton tetramers in solution (data not shown).

Further confirmation for the identity of these two proteins was achieved by demonstrating that the rat liver HDP isolated by ss DNA cellulose chromatography and the rat liver LDH isolated by N^6-(6-aminohexyl)-AMP-Sepharose affinity chromatography both had similar specific activities when assayed for either DNA binding or LDH activity. In addition, reverse-phase HPLC tryptic peptide maps (Fig. 4) were identical for the two proteins, suggesting that there were no minor sequence or posttranslational differences between them.

DISCUSSION

Based on several independent criteria, it now seems certain that the ss DNA binding HDP previously isolated from rat liver (5, 6) is actually LDH. Therefore, it is referred to in this *Discussion* as HDP/LDH. It is not, however, yet certain if there is any physiological significance attached to the finding that it can bind reasonably tightly and specifically to ss DNA. The purification procedure that was used for the rat liver HDP is based on that of Herrick and Alberts (21), which requires that the protein pass through a double-stranded DNA cellulose column, bind to a ss DNA cellulose column connected in tandem, and resist elution from this latter column by dextran sulfate. This latter step should elute proteins that are bound nonspecifically by electrostatic interactions with the polyanionic backbone of ss DNA. While HDP/LDH behaves much like other well-characterized prokaryotic ss DNA binding proteins do on these affinity supports, it nonetheless is eluted at a much lower salt concentration. It can be eluted from ss DNA-cellulose with 0.15 M NaCl compared with the >1.0 M salt concentrations that are required to quantitatively elute the bacteriophage T4 (22) or *E. coli* ss DNA binding proteins (23). In this respect HDP/LDH is similar to glyceraldehyde-3-phosphate dehydrogenase, which also was identified as a ss DNA binding protein and that also can be eluted from ss DNA-cellulose by 0.15 M NaCl (24). Further support for the relatively low ss DNA binding affinity of HDP/LDH derives from studies on the lowering of the thermal melting temperature of poly[d(A-T)·d(A-T)] by HDP. Reddigari has shown that in 0.05 M NaCl, HDP lowers the melting temperature of this polynucleotide by about 10°C (25), compared to about 25°C for the *E. coli* ss DNA binding protein. An analysis (26) of this data suggests that HDP/LDH binds ss DNA with an affinity that is between 10^6 and 10^7 M^{-1} in 0.05 M NaCl (27), which compares with a value of 10^{14} M^{-1} for *E. coli* SSB under the same conditions (unpublished data). In addition, several other proteins, including the P-1 protein (protocollagen precursor) from fibroblasts (28), α-1-antichymotrypsin (29), and three serum proteins involved in complement activation: C3DP (30), factor B (31), and β1H, (32) that apparently have no *in vivo* role in DNA metabolism nonetheless bind to ss DNA-cellulose. Taken together, these data indicate that some care needs to be exercised in ascribing *in vivo* functions simply on the basis of binding to ss DNA-cellulose (2) or acting as a helix-destabilizing protein *in vitro*. It might be

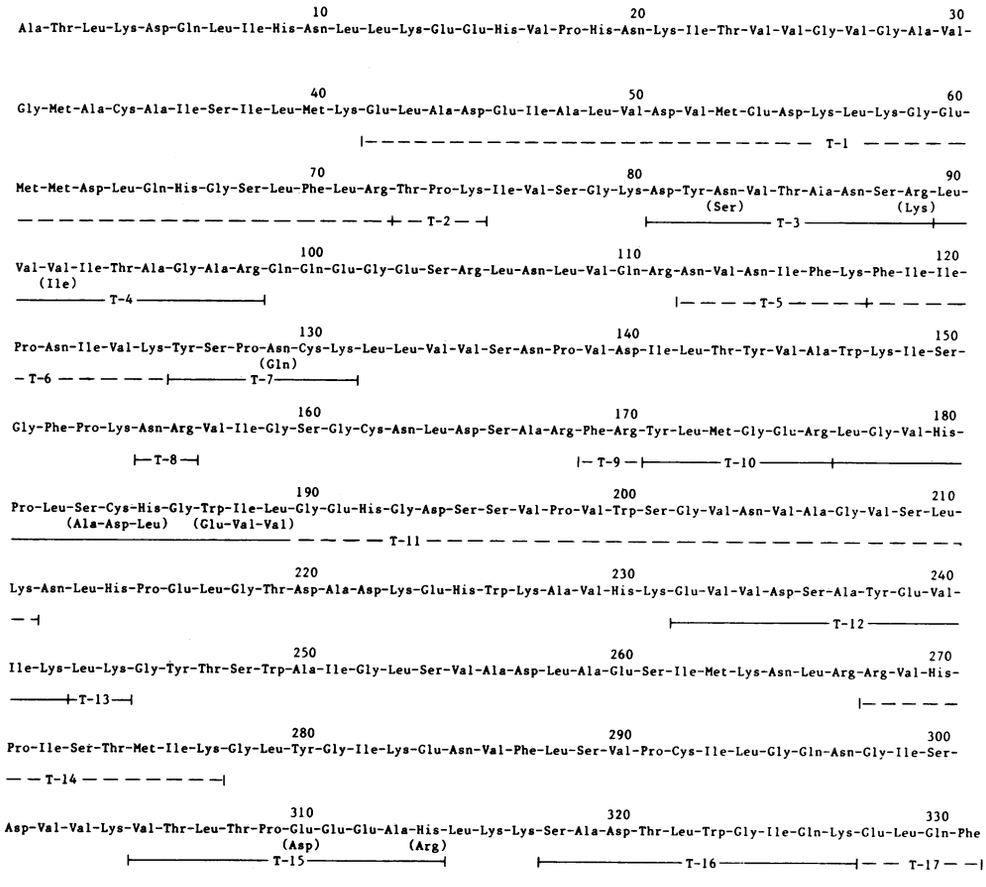

FIG. 3. Amino acid sequence homology between porcine M chain LDH and the rat liver HDP. Underneath the porcine LDH sequence (20) are those tryptic peptides from the rat liver ss DNA binding protein that were matched to the LDH sequence by amino acid composition (dashed line) or by solid-phase sequencing (solid line). Amino acid substitutions in the rat LDH are indicated in parentheses under the porcine LDH sequence.

advisable to take as an initial criterion that an otherwise unidentified protein should not be considered to function primarily as a single-stranded nucleic acid binding protein *in vivo* unless it requires at least 0.5 M NaCl to elute it from ss DNA-cellulose and/or has an affinity of at least 10^7 M^{-1} for ss DNA under physiological salt concentrations. It is also clearly justified to obtain some primary sequence data on any such protein and then to have it searched against the Protein Data Base.

The ss DNA binding that both glyceraldehyde-3-phosphate dehydrogenase and HDP/LDH exhibit seems to occur in a region that is close to their respective coenzyme binding sites. Thus, NAD strongly inhibits the binding of glyceraldehyde-3-phosphate dehydrogenase to ss DNA (24), and NADH prevents HDP/LDH binding to ss DNA (6). In addition, the tyrosine residue that we have identified in HDP/LDH as being involved in ss DNA binding is conserved in every LDH enzyme that has been sequenced (33). X-ray crystallography studies further indicate that tyrosine-238 is located very close to a loop of the polypeptide chain (containing approximately residues 98–110) that undergoes a considerable conformational change upon substrate and coenzyme binding (33). In the apoenzyme this loop extends outward into the solvent, whereas in the enzyme–lactate–NAD ternary complex, the loop extends over the active site and prevents the entry or release of coenzyme (33). In this latter conformation, this loop is close to tyrosine-238 (which corresponds to tyrosine-237 in ref. 33). Diazotization of this tyrosine residue with sulfanilic acid or iodination abolishes LDH activity (34, 35). On the other hand, nitration of tyrosine-238 seems to have a more pronounced effect on the ability of HDP/LDH to act as a ss DNA binding protein *in vitro* than on its ability to act as a dehydrogenase. The specific activity of the nitrated LDH is only about 25% less than that of the native enzyme (36). With respect to these studies, it is interesting that LDH is one of three glycolytic enzymes that are phosphorylated at tyrosine in cells transformed by Rous sarcoma virus (37). Recently, tyrosine-238 has been identified as the site of this phosphorylation (38). It is not yet known what effect this phosphorylation has on either the dehydrogenase or ss DNA binding "activities" of LDH.

At present, we are unable to reconcile the homology between rat HDP and LDH-5 described herein with our earlier study (7), which (*i*) reported that a single component in ss DNA binding proteins of *Drosophila melanogaster* embryos cross-reacted with an antiserum against rat HDP and (*ii*) showed localization of this immunoreactive HDP in transcriptionally active puffs on polytene chromosomes. Similarities in the chromosomal patterns of HDP distribution and RNA polymerase distribution reported by others (39) had led us to implicate HDP in some transcriptional role. It should be noted that a protein factor purified from the HeLa cell extract, which stimulates specific transcription *in vitro*, exhibits some properties strikingly similar to those of actin (40), and epidermal growth factor receptor exhibits partial DNA topoisomerase II activity (41). In the aggregate, these data raise the intriguing possibility that some proteins may be capable of alternate biological activities. However, this idea must await additional evidence from comparison of isolated genes and/or mutants of respective activities to become a biological reality.

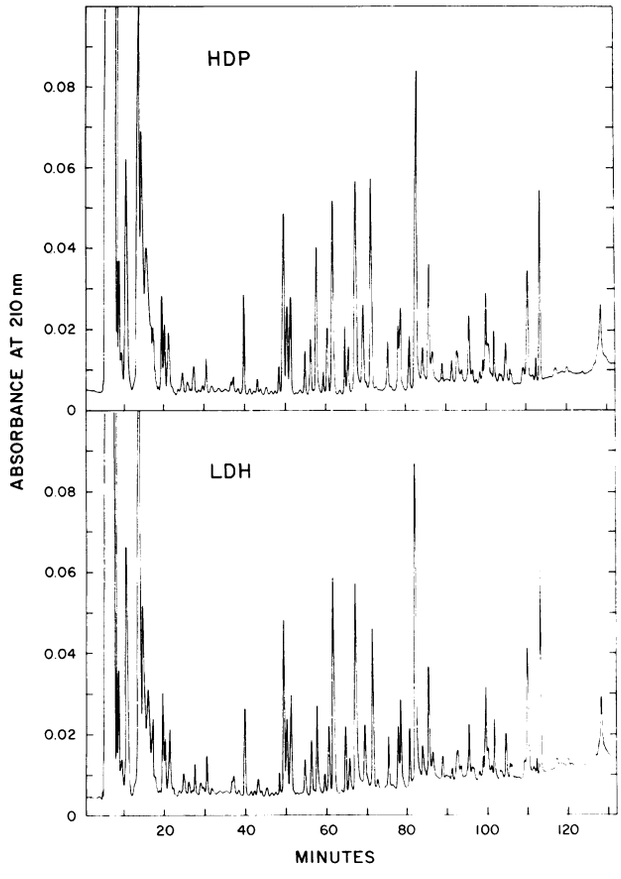

FIG. 4. HPLC separation of tryptic peptides from 2.0 nmol of rat liver HDP purified by ss DNA-cellulose (*Top*) or of rat liver LDH purified by N^6-(6-aminohexyl)-AMP-Sepharose affinity chromatography. The Waters C_{18} column was eluted as described.

We especially thank Mary LoPresti, who assisted with the protein chemistry studies, and Sangram Sisodia, who provided purified rat LDH. This work was supported by grants from National Institutes of Health (CA1138 to G.L.P. and GM31539 to K.R.W.) and National Science Foundation (PCM-8310692 to G.L.P.).

1. Kowalczykowski, S. C., Bear, D. G. & von Hippel, P. H. (1981) in *The Enzymes*, ed. Boyer, P. (Academic, New York), Vol. 14a, pp. 373–444.
2. Williams, K. R. & Konigsberg, W. H. (1981) in *Gene Amplication and Analysis*, eds. Chirikjian, J. G. & Papas, T. S. (Elsevier/North-Holland, New York), Vol. 2, pp. 475–508.
3. Williams, K. R. & Konigsberg, W. H. (1983) in *Bacteriophage T4*, eds. Mathews, C. K., Kutter, E. M., Mosig, G. & Berget, P. B. (American Society for Microbiology, Washington, D.C.), pp. 82–89.
4. Karpel, R. L. (1981) in *Gene Amplification and Analysis*, eds. Chirikjian, J. G. & Papas, T. S. (Elsevier/North-Holland, New York), Vol. 2, pp. 509–536.
5. Patel, G. L. (1978) *J. Supramol. Struct.* **8** Suppl. 2, 323 (abstr.).
6. Patel, G. L., Reddigari, S., Williams, K. R., Baptist, E., Thompson, P. E. & Sisodia, S. (1984) in *Growth, Cancer, and the Cell Cycle*, eds. Skehan, P. & Friedman, S. J. (Humana, Clifton, NJ), pp. 41–58.
7. Patel, G. L. & Thompson, P. E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6749–6753.
8. O'Connor, T. P. & Coleman, J. E. (1983) *Biochemistry* **22**, 3375–3381.
9. Prigodich, R. V., Casas-Finet, J., Williams, K. R., Konigsberg, W. H. & Coleman, J. E. (1984) *Biochemistry* **23**, 522–529.
10. Merrill, B. M., Williams, K. R., Chase, J. W. & Konigsberg, W. H. (1984) *J. Biol. Chem.* **259**, 10850–10856.
11. Helene, C. & Maurizot, J. C. (1981) *CRC Crit. Rev. Biochem.* **10**, 213–258.
12. Alberts, B. & Herrick, G. (1971) *Methods Enzymol.* **21D**, 198–217.
13. Lee, C.-Y., Yuan, J. H. & Goldberg, E. (1982) *Methods Enzymol.* **89**, 351–358.
14. Riggs, A. D., Bourgeois, S., Newby, R. & Cohn, M. (1968) *J. Mol. Biol.* **34**, 365–368.
15. Sokolovsky, M., Riordan, J. F. & Vallee, B. L. (1966) *Biochemistry* **5**, 3582–3589.
16. Fanning, T. G. (1975) *Biochemistry* **14**, 2512–2520.
17. Hemmings, H. C., Williams, K. R., Konigsberg, W. H. & Greengard, P. (1984) *J. Biol. Chem.* **259**, 14486–14490.
18. Anderson, R. A., Nakashima, Y. & Coleman, J. E. (1975) *Biochemistry* **14**, 907–917.
19. Anderson, R. A. & Coleman, J. E. (1975) *Biochemistry* **14**, 5485–5491.
20. Kiltz, H., Keil, W., Griesbach, M., Petry, K. & Meyer, H. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* **358**, 123–127.
21. Herrick, G. & Alberts, B. (1976) *J. Biol. Chem.* **251**, 2124–2132.
22. Alberts, B. M. & Frey, L. (1970) *Nature (London)* **227**, 1313–1318.
23. Molineux, I. J., Friedman, S. & Gefter, M. L. (1974) *J. Biol. Chem.* **249**, 6090–6098.
24. Perucho, M., Salas, J. & Salas, M. L. (1977) *Eur. J. Biochem.* **81**, 557–562.
25. Reddigari, S. R. (1983) Dissertation (University of Georgia, Athens).
26. Crothers, D. (1971) *Biopolymers* **10**, 2147–2160.
27. Jensen, D. E. & von Hippel, P. H. (1976) *J. Biol. Chem.* **251**, 7198–7214.
28. Tsai, R. L. & Green, H. (1972) *Nature (London) New Biol.* **237**, 171–173.
29. Siddiqui, A. A., Hughes, A. E., Davies, R. J. H. & Hill, J. A. (1980) *Biochem. Biophys. Res. Commun.* **95**, 1737–1742.
30. Schroder, C. & Weinblum, D. (1981) *Hoppe-Seyler's Z. Physiol. Chem.* **362**, 1599–1607.
31. Gardner, W. D., Haselby, J. A. & Hoch, S. O. (1980) *J. Immunol.* **124**, 2800–2806.
32. Gardner, W. D., White, P. J. & Hoch, S. O. (1980) *Biochem. Biophys. Res. Commun.* **94**, 61–67.
33. Eventoff, W., Rossmann, M. G., Taylor, S. S., Torff, H., Meyer, H., Keil, W. & Kiltz, H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2677–2681.
34. Pfleiderer, G. & Jeckel, D. (1967) *Eur. J. Biochem.* **2**, 171–175.
35. di Sabato, G. (1965) *Biochemistry* **4**, 2288–2296.
36. Parker, D. M., Jeckel, D. & Holbrook, J. J. (1982) *Biochem. J.* **201**, 465–471.
37. Cooper, J. A., Reiss, N. A., Schwartz, R. J. & Hunter, T. (1983) *Nature (London)* **302**, 218–223.
38. Cooper, J. A., Esch, F. S., Taylor, S. S. & Hunter, T. (1984) *J. Biol. Chem.* **259**, 7835–7841.
39. Jamrich, M., Greenleaf, A. L. & Bautz, E. K. F. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2079–2083.
40. Egly, J. M., Miyamoto, N. G., Moncollin, V. & Chambon, P. (1984) *EMBO J.* **2**, 2362–2372.
41. Mroczkowski, B., Mosig, G. & Cohen, S. (1984) *Nature (London)* **309**, 270–273.