Identification of the mouse low-salt-eluting single-stranded DNA-binding protein as a mammalian lactate dehydrogenase-A isoenzyme

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A 36000- M_r protein purified from mouse myeloma on the basis of selective binding to a single-stranded DNA (ssDNA)–cellulose column has been identified as the lactate dehydrogenase A (LDH-A) subunit. A homogeneous preparation of this mouse myeloma ssDNA-binding protein, termed the ‘low-salt-eluting protein’, was found to possess LDH activity, and rabbit antiserum prepared against this protein was shown to cross-react with purified 36000- M_r LDH-A subunits from mouse and bovine sources. In addition, bovine and human LDH-A₄ isoenzymes were shown to be capable of binding ssDNA. These enzymic and immunological identities with LDH-A were not observed with purified helix-destabilizing protein 1 from mouse myeloma. A model for ssDNA–LDH binding is discussed.

INTRODUCTION

Many ssDNA-binding proteins from eukaryotes and prokaryotes [1] have been purified by selective binding to ssDNA, as applied by Herrick & Alberts [2]. These proteins pass through a native (double-stranded) DNA–cellulose column, are bound to a denatured (single-stranded) DNA–cellulose column, are resistant to a dextran sulphate wash, and are eluted with a NaCl gradient. From calf thymus, three different ssDNA-binding proteins were isolated and well characterized: (a) low-salt (0.15 M-NaCl) eluting basic protein of 33 kDa, (b) medium-salt (0.4 M-NaCl) eluting helix-unwinding protein (UP-1) of heterogeneous molecular masses around 24 kDa, and (c) high-salt (2.0 M-NaCl) eluting acidic protein fraction of 33 kDa [2–4]. From mouse myeloma cells, a ssDNA-binding 36 kDa protein, eluted at 0.15 M-NaCl, and a helix-destabilizing protein (HDP-1) of heterogeneous molecular masses ranging from 24 to 33 kDa were purified [5].

In mammals the five isoenzymes of tetrameric LDH are found in various proportions among different somatic tissues and are formed *in vivo* by combination of the A and B subunits, whereas the homotetrameric LDH-C₄ isoenzyme is present only in mature testes and sperm [6]. The LDH-5 (A₄, skeletal muscle), LDH-1 (B₄, heart muscle) and LDH-X (C₄, testes and sperm) isoenzymes possess distinct physicochemical, enzymic and immunological properties [7, 8]. The three genes encoding the LDH-A, B and C isoenzymes are believed to have originated from an ancestral gene during the course of evolution [6, 9].

In an abstract by Williams *et al.* [10], a rat liver DNA-binding protein of 30000 Da was reported to have partial sequences homologous with porcine LDH isoenzyme. This prompted us to investigate the possible relation between eukaryotic ssDNA-binding proteins and

mammalian LDH isoenzymes, the subunits of which have approximately the same molecular masses as several of the ssDNA-binding proteins. We have found that mouse myeloma low-salt-eluting 36 kDa protein, but not HDP-1, shows LDH activity and cross-reacts immunologically with mouse and bovine LDH-A₄ isoenzymes, and that bovine and human LDH-A₄ isoenzymes indeed bind ssDNA on Western blot and filter-disc binding assays. A model for ssDNA–LDH binding is proposed, and possible identities of mammalian LDH-A, B and C isoenzymes [6–9] with the previously reported eukaryotic DNA-binding proteins [1–5, 11–13] are discussed.

MATERIALS AND METHODS

Materials

The low-salt-eluting ssDNA-binding 36 kDa protein and HDP-1 were purified from mouse myeloma cells [5] and rabbit antiserum against this 36 kDa protein was obtained essentially as previously described [14]. Mouse LDH-A₄ isoenzyme was purified as reported [15]. The LDH-A₄ isoenzymes from bovine muscle and human placenta, as well as the LDH-B₄ isoenzymes from bovine heart and human red blood cells, were obtained from Sigma. *Escherichia coli* ssDNA-binding protein (SSB) was from Worthington. Human LDH-A cDNA used in Western blot binding was labelled with ³²P by nick-translation [16]. The ¹⁴C-labelled *E. coli* DNA used in filter binding assays was from Amersham International. The Vectastaining ABC kit from Vector Labs was used for immunodetection.

SDS/polyacrylamide-gel electrophoresis

Discontinuous polyacrylamide slab gels were run using the Tris/glycine/SDS buffer as described by Laemmli [17] to separate proteins of different molecular masses. The

Abbreviations used: ssDNA, single-stranded DNA; SSB, ssDNA-binding protein; UP, helix-unwinding protein; HDP, helix-destabilizing protein; LDH, lactate dehydrogenase.

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Fig. 1. LDH enzymic activity of mouse myeloma ssDNA-binding proteins

The low-salt-eluting ssDNA-binding 36 kDa protein (lane 1) and helix-destabilizing 24 kDa protein (HDP-1, lane 2) purified from mouse myeloma cells were electrophoresed on alkaline native polyacrylamide gel, and the gel was stained for LDH enzymic activity by the standard procedure provided by Sigma.

Fig. 2. SDS/polyacrylamide-gel electrophoresis followed by either protein staining (a) or immunodetection (b) of mouse myeloma ssDNA-binding proteins, and mouse and bovine LDH isoenzymes

After SDS/polyacrylamide-gel electrophoresis, the proteins were either stained with Coomassie Blue (a) or Western blotted onto a nitrocellulose filter and immunodetected (b) with rabbit antiserum prepared against mouse myeloma 36 kDa protein. Lanes 1, mouse LDH-A₄ isoenzyme; lanes 2, mouse myeloma 36 kDa protein (small amount loaded); lanes 3, mouse myeloma HDP-1 protein; lanes 4, bovine LDH-A₄ isoenzyme; lanes 5, bovine LDH-B₄ isoenzyme.

proteins were either stained with Coomassie Blue R250 or Western blotted onto a nitrocellulose filter for detecting immunological cross-reactivity with rabbit antiserum prepared against mouse myeloma 36 kDa protein [18].

Fig. 3. LDH enzymic and ssDNA-binding activities of bovine and human LDH isoenzymes

Bovine and human LDH-A₄ and LDH-B₄ isoenzymes were electrophoresed on a alkaline native polyacrylamide gel, and either stained for LDH activity (a) or Western blotted on nitrocellulose filter for ssDNA binding using the denatured human LDH-A cDNA labelled with ³²P by nick-translation (b). Similar binding results on a Western blot were also obtained with the denatured DNA probe of *Drosophila* alcohol dehydrogenase gene (results not shown). Lanes 1, human LDH-A₄; lanes 2, human LDH-B₄; lanes 3, bovine LDH-A₄; lanes 4, bovine LDH-B₄.

Alkaline native polyacrylamide gel electrophoresis

The LDH isoenzymes and ssDNA-binding proteins were electrophoresed on alkaline polyacrylamide gels [19], and either stained for LDH activity or Western blotted onto nitrocellulose filters [20] for ssDNA-binding using ³²P-labelled human LDH-A cDNA [16].

Filter-disc protein-ssDNA binding assay

The filter-disc protein-ssDNA binding assay using ¹⁴C-labelled *E. coli* DNA was also used to measure the relative binding of bovine and human LDH-A₄ and LDH-B₄ isoenzymes along with *E. coli* SSB protein [21].

RESULTS

Identity of low-salt-eluting ssDNA-binding 36 kDa protein with LDH-A subunit

The low-salt-eluting ssDNA-binding 36 kDa protein and HDP-1 of heterogeneous molecular masses from

Table 1. ssDNA-binding activity of mammalian LDH isoenzymes and *E. coli* SSB protein

The average binding (c.p.m.) of duplicate samples is given in the Table; similar results were obtained in other experiments.

Protein	Monomeric molecular mass (kDa)	Amount used (μ g)	Binding (c.p.m.)
Bovine LDH-A ₄	36	10	2234
Bovine LDH-B ₄	36	10	739
Human LDH-A ₄	36	10	4005
Human LDH-B ₄	36	10	878
Bovine serum albumin	68	10	53
<i>E. coli</i> SSB	19	1	11645

mouse myeloma cells [5] were electrophoresed on a native polyacrylamide gel and stained for LDH enzymic activity. The result (Fig. 1) clearly showed that the 36 kDa protein, but not HDP-1 protein, exhibited LDH enzymic activity. Mouse myeloma 36 kDa protein and HDP-1 protein, mouse LDH-A₄, bovine LDH-A₄ and B₄ isoenzymes were run on SDS/polyacrylamide gels, and these proteins were either stained with Coomassie Blue (Fig. 2a) or transferred on nitrocellulose filters and immunodetected with rabbit antiserum prepared against mouse myeloma ssDNA-binding 36 kDa protein (Fig. 2b). These results indicate that mouse myeloma low-salt-eluting ssDNA-binding 36 kDa protein has the same apparent molecular mass and cross-reacts immunologically with mouse and bovine LDH-A subunits. Bovine LDH-B subunit appeared to have slightly lower mobility and showed weak cross-reactivity with mouse myeloma 36 kDa protein. The predominant species of mouse HDP-1 protein had molecular mass lower than 36 kDa and did not show any immunological cross-reactivity with antiserum prepared against the mouse low-salt-eluting ssDNA-binding 36 kDa protein, confirming that mouse myeloma HDP-1 and 36 kDa proteins have quite different structures [5].

The ssDNA activity of bovine and human LDH-A₄ isoenzymes

Bovine and human LDH-A₄ and B₄ isoenzymes were electrophoresed on native polyacrylamide gels and stained for LDH enzymic activity (Fig. 3a). These same isoenzymes on duplicate gels were Western blotted onto a nitrocellulose filter for ssDNA binding, and both bovine and human LDH-A₄ isoenzymes did bind to the denatured human LDH-A cDNA labelled with ³²P by nick-translation (Fig. 3b). The relative ssDNA-binding activity of bovine and human LDH-A₄ and LDH-B₄ isoenzymes in comparison with *E. coli* SSB protein was measured by filter-disc binding assay using the denatured, ¹⁴C-labelled *E. coli* DNA, and the results are given in Table 1. Bovine and human LDH-A₄ isoenzymes did show significant ssDNA binding, although their binding affinities were not as strong as that of *E. coli* SSB protein. LDH-B₄ isoenzymes from both bovine and human also exhibited weak ssDNA binding.

DISCUSSION

In this study, we have shown that the mouse myeloma 36 kDa low-salt-eluting ssDNA-binding protein, but not HDP-1 [5], is identical with mouse LDH-A subunit [15,

22]. The calf thymus low-salt-eluting 33 kDa basic protein [2] and the P8 proteins (eluted from an ssDNA-cellulose column at 0.15 M-NaCl) from mouse 3T3 cells and human diploid fibroblasts [11] are probably LDH-A isoenzymes also. The ssDNA-binding affinity of these proteins and of bovine and human LDH-A₄ isoenzymes appears to be much lower than that of *E. coli* SSB protein (Table 1) and of mouse HDP-1 [5]. While this manuscript was in preparation, Williams *et al.* [23] described the identification of a rat liver ssDNA-binding protein of 30000 Da as LDH-5 (LDH-A₄), and the rat liver ssDNA-binding protein/LDH has a ssDNA-binding constant of 10⁻⁶ and 10⁻⁷ M⁻¹, compared with a value of 10⁻¹⁴ M⁻¹ for *E. coli* SSB in 0.05 M-NaCl. Williams *et al.* [24] reported that the calf thymus UP-1 protein and mouse myeloma HDP-1 protein are homologous [24] and their structures are quite different from those of bovine and mouse LDH-A isoenzymes [9, 22]. Therefore, the rat ssDNA-binding/LDH protein is really a low-salt-eluting ssDNA-binding LDH-A protein and its molecular mass should be 36000 Da, not 30000 Da as previously estimated [23]. Although the physiological significance *in vivo* of the ssDNA-binding activities of low-salt-eluting ssDNA-binding 36 kDa/LDH-A proteins remains to be illustrated, it has been suggested that these proteins might play some roles in DNA transcription, or RNA processing and transport [23]. The calf thymus UP-1 and mouse myeloma HDP-1 proteins might have functions *in vivo* analogous to that of bacteriophage T4 gene 32 HDP protein and *E. coli* SSB protein in DNA replication, repair and recombination [1, 25, 26].

The ssDNA-binding property *in vitro* of the LDH-A proteins was shown to be inhibited by NADH and by the specific modification of tyrosine at residue 238 [23], indicating that the coenzyme-binding site and the loop peptide of LDH isoenzyme may be involved in ssDNA binding. Since the structure of mammalian LDH-A proteins is well established [8], it is easy to envision that the ssDNA may be bound to the dinucleotide subdomain when the loop of LDH apoenzyme is open. The conserved basic amino acids, such as Lys-56 and Arg-98, might be directly involved in ssDNA binding through electrostatic interactions. The conserved aromatic amino acids such as Tyr-83 and Tyr-238 might also be important in ssDNA binding through hydrogen-bonding or intercalation. On the basis of the very conserved structure of vertebrate LDH-A (muscle), LDH-B (heart) and LDH-C (testis) isoenzymes [9], this model for LDH protein-ssDNA binding predicts that all of mammalian LDH isoenzymes (A₄, A₃B₁, A₂B₂, A₁B₃, B₄, C₄) might bind to ssDNA. It

would be of great interest if calf thymus high-salt-eluting acidic 33 kDa protein [2] and mouse ascites ssDNA-binding protein of 30–35 kDa [12] turned out to be the LDH-B isoenzyme, and if the rat spermatocyte R-protein of 33–35 kDa [13] was the LDH-C isoenzyme [9]. It may be noted that mouse and human LDH-A genes have been isolated [22, 27], and that the full-length cDNA clones of rat and human LDH-A isoenzymes [16, 28] are available for further studies by site-directed mutagenesis on the effects of structural modifications of LDH-A proteins on their ssDNA-binding and LDH enzymic activities.

We thank Dr. B. H. Judd for his interest and support to this project, and Drs. F.-Z. Chung, R. K. Hamatake and A. Sugino for helpful discussion and reading the manuscript.

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Received 16 October 1985; accepted 13 November 1985