CMLS, Cell. Mol. Life Sci. 58 (2001) 902–920
1420-682X/01/070902-19 $ 1.50 + 0.20/0
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CMLS Cellular and Molecular Life Sciences

Review

Multifunctional α-enolase: its role in diseases

V. Pancholi

Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, 1230 York Avenue, New York (New York 10021, USA), Fax +1 212 327 7584, e-mail: panchov@rockvax.rockefeller.edu

Received 6 December 2000; received after revision 24 January 2001; accepted 26 January 2001

Abstract. Enolase, a key glycolytic enzyme, belongs to a novel class of surface proteins which do not possess classical machinery for surface transport, yet through an unknown mechanism are transported on the cell surface. Enolase is a multifunctional protein, and its ability to serve as a plasminogen receptor on the surface of a variety of hematopoetic, epithelial and endothelial cells suggests that it may play an important role in the intravascular and pericellular fibrinolytic system. Its role in systemic and invasive autoimmune disorders was recognized only very recently. In addition to this property, its ability to function as a heat-shock protein and to bind cytoskeletal and chromatin structures indicate that enolase may play a crucial role in transcription and a variety of pathophysiological processes.

Key words. Enolase; autoimmunity; rheumatic fever; SLE.

Introduction

Glycolytic enzymes (including α-enolase) are considered rather 'dull' enzymes because of their conserved nature through millions of year. They have been stamped as enzymes with no sophisticated regulatory properties, and the ones which just turn over substrates in either direction as steady-state concentrations fluctuate in response to other regulatory pressures. Despite this boring attitude, these enzymes are perhaps the most well characterized proteins, and their detailed structural analyses have provided a strong basis for understanding some of the fundamental aspects of biochemistry, especially the evolution of glycolysis and the process of life itself [1, 2]. Recent findings have shown that these enzymes perform several functions in addition to their innate glycolytic function and play an important role in several biological and pathophysiological processes [3, 4]. This, in turn, requires us to look them as old proteins with new faces. Especially in view of several reports showing a marked increase in the titer of anti-enolase antibodies in a variety of autoimmune diseases, the purpose and emphasis of this review is to provide a fresh look at one of the key glycolytic enzymes, α-enolase, and elucidate its structure-function relationship in a variety of biological and disease processes.

Definition and early history

Enolase was discovered in 1934 by Lohman and Mayerhof [5] while they were studying the conversion of 3-phosphoglycerate to pyruvate in muscle extracts. The enzyme α-enolase (2-phospho-D-glycerate hydrolyase, EC 4.2.1.11) is a metalloenzyme that catalyzes the dehydration of 2-phospho-D-glycerate (PGA) to phosphoenolpyruvate (PEP) in the forward or catabolic direction in the second half of the Emden Mayerhoff-Parnas glycolytic pathway [6]. In the reverse reaction (anabolic pathway) which occurs during gluconeogenesis, the same enzyme catalyzes hydration of PEP to PGA (phosphopyruvate hydratase) (fig. 1). Enolase has an absolute requirement for certain divalent metal ions for its activity. Magnesium is the natural cofactor, in the presence of which enolase imparts its highest activity, and hence it is also classified among the enzymes called metal-activated metalloenzy-

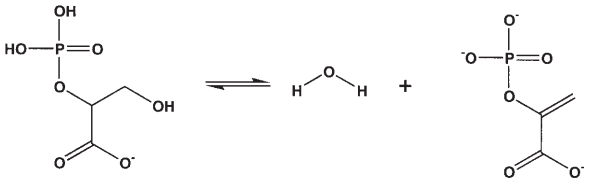


Figure 1. Enolase reaction.

mes. Enolase reaction occupies a key position in the metabolic pathway of fermentation in general and the glycolytic pathway, in particular, and hence this enzyme is ubiquitously present in abundance in the biological world [6].

Properties and distribution

Enolase is one of the most abundantly expressed cytosolic proteins in many organisms. More recently it was also found on the cell surface [7–9]. In vertebrates, the enzyme occurs as three isoenzymes: α-enolase is found in a variety of tissues including liver, whereas β-enolase is almost exclusively found in muscle tissues and γ-enolase is found in neuron and neuroendocrine tissues [10]. All enolases are made up of two identical subunits, and have a molecular weight in the range of 82,000–100,000 Da [6, 11]. In mammals and humans, there are three independent genetic loci, α, β and γ, that encode for three isozymes [10, 12, 13]. Several earlier studies have determined from their chromatographic and immunological studies that neuron/brain – specific enolase can be found in heterodimeric forms such as $\alpha\alpha$, $\alpha\beta$, $\beta\beta$, $\alpha\gamma$ and $\gamma\gamma$ [12–14]. As yet $\beta\gamma$ heterodimeric isoenzyme has not been found, although experimentally it has been isolated from its individual homodimeric counterparts [15]. Rider and Taylor [15, 17] and Merkulova et al. [16] determined that the proportions of isoenzymes $\alpha\alpha$, $\beta\beta$ and $\alpha\beta$ in heart and skeletal muscle of rat change during embryonic development. In both tissues, $\alpha\alpha$ isoenzyme predominates in the fetus: however, as development progresses, this isoenzyme is replaced by $\alpha\beta$ and $\beta\beta$ types in adult heart and by $\beta\beta$ type in adult muscle. The researchers also showed that the $\alpha\alpha$ isoform occurs in liver, brain, spleen, adipose tissue and kidney.

Metal ions and enolase activity

The metal ion requirement of the enzyme was first observed independently by Warburg and Christian [18] and Utter and Werkman [19]. However, only the former group first purified and crystallized enolase from yeast and carried out the kinetic study. Since then several groups have studied the importance and mechanisms of metal ion activation of the enolase enzyme (see review [6, 11]). Using equilibrium dialysis and electron spin resonance measurement techniques, a total of six divalent metal ions – magnesium, manganese, zinc, cadmium, cobalt and nickel – were found to activate enolase [20]. Among these, although magnesium is a naturally occurring metal activator, the binding of enolase with magnesium is much weaker than that with zinc [20, 21].

Thus, according to the definition of Valle [22], enolase qualifies as a metal-ion-activated enzyme complex rather then a typical metalloenzyme in which the metal is firmly bound. The role of Mg^{++} in the enolase activity is twofold since it possesses two types of binding sites which contribute to catalysis [23]. The first site is called conformational. It induces conformational changes in the active site and enables binding of a substrate or its analogues (Metalloenzyme). Once firmly bound, the second magnesium ion binds to the second site and serves as a required component of the catalytic apparatus. The relative activation strength profile of binding of metal ions in the enolase activity is $Mg^{2+} > Zn^{2+} > Mn^{2+} > Fe(II)^{2+} > Cd^{2+} > Co^{2+}$, Ni^{2+}, Sm^{3+}, Tb^{3+} and most other divalent metal ions [6, 11, 20]. Irrespective of which of these metal ions binds, the studies on yeast enolase have shown that metal-ion binding in the catalytic site is always weaker when compared with that in the conformational site. Thus, although Ca^{2+}, Tb^{2+} and Sm^{3+} do not activate enolase, they bind to enolase much tighter than Mg^{2+} [21].

Primary and secondary structure of α-enolase

It has been known since 1971 that enzymatically α-enolase exists in a dimeric form [6]. In the late 80s and early 90s, the reports from Lebioda's [24–29] and Reed's labs [30–33] defined the X-ray crystal structure of yeast enolase and confirmed that α-enolase is composed of two identical subunits facing each other in an antiparallel fashion. In this subunit interaction, the N-terminal of one subunit faces the C-terminal of the second in such a way that the residue Glu20 forms an ionic pair with Arg414. Thus far, amino acid sequences of more than 50 enolases are available. In the present review, a comparison of amino acid sequences of enolase enzymes from 39 representative species, as carried out by the Custal method, is shown in figure 2. Despite these sequence differences, as is true for most glycolytic enzymes, enolase is highly conserved (fig. 3). This comparison shows 40–90% identity between enolases from two different species (figs. 2–4). Each subunit of enolase is made up of two domains: the smaller N-terminal and the larger C-terminal domains. The N-terminal domain has a $\beta_3\alpha_4$ topology. The second domain has the eightfold $\beta\alpha$ barrel structure with $\beta\beta\alpha\alpha(\beta\alpha)_6$ topology (fig. 5). The residues that participate (see the mechanisms of enolase activity, below) in catalytic activity are conserved throughout. The conservation of flanking residues on either side of these ca-

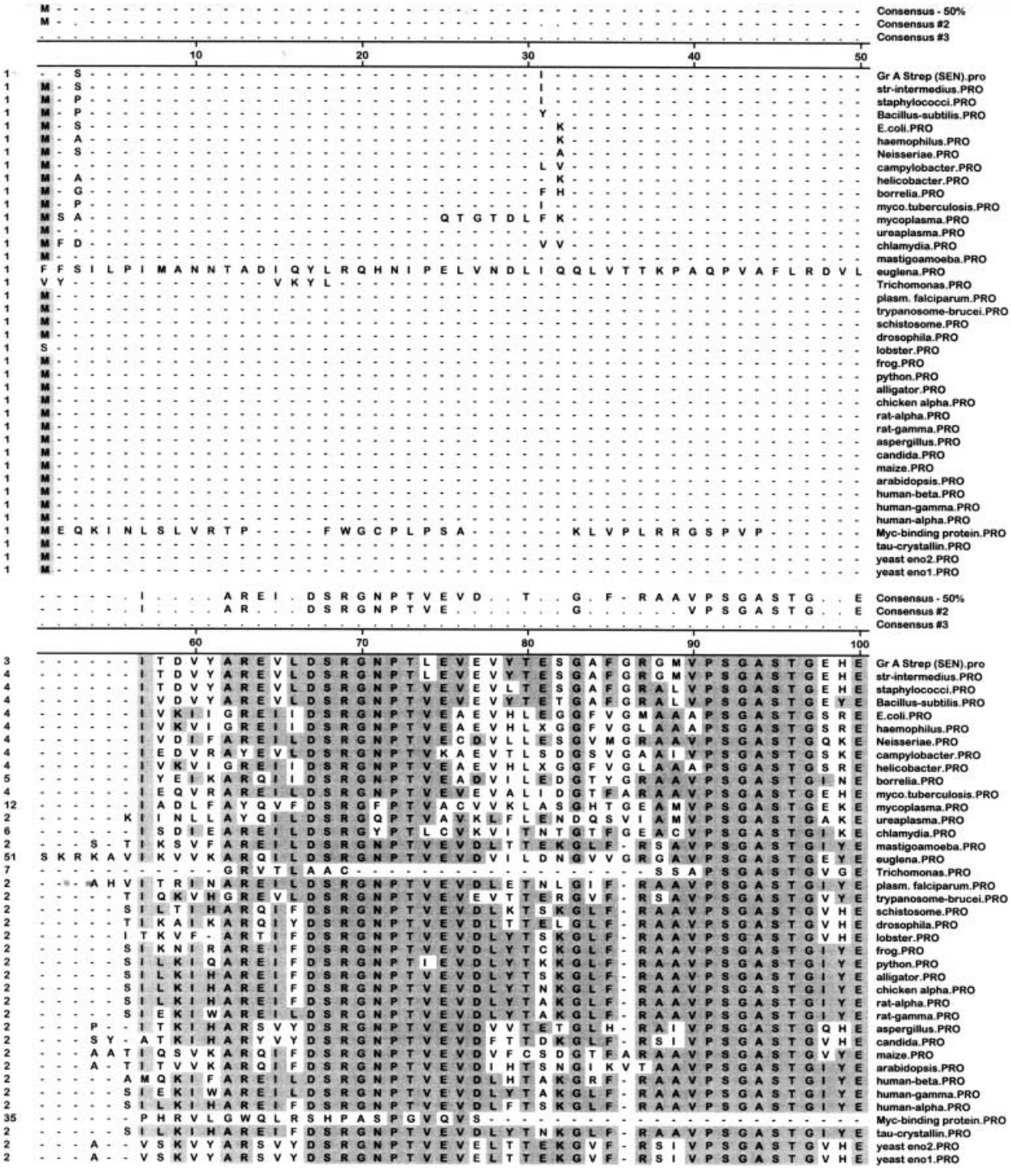
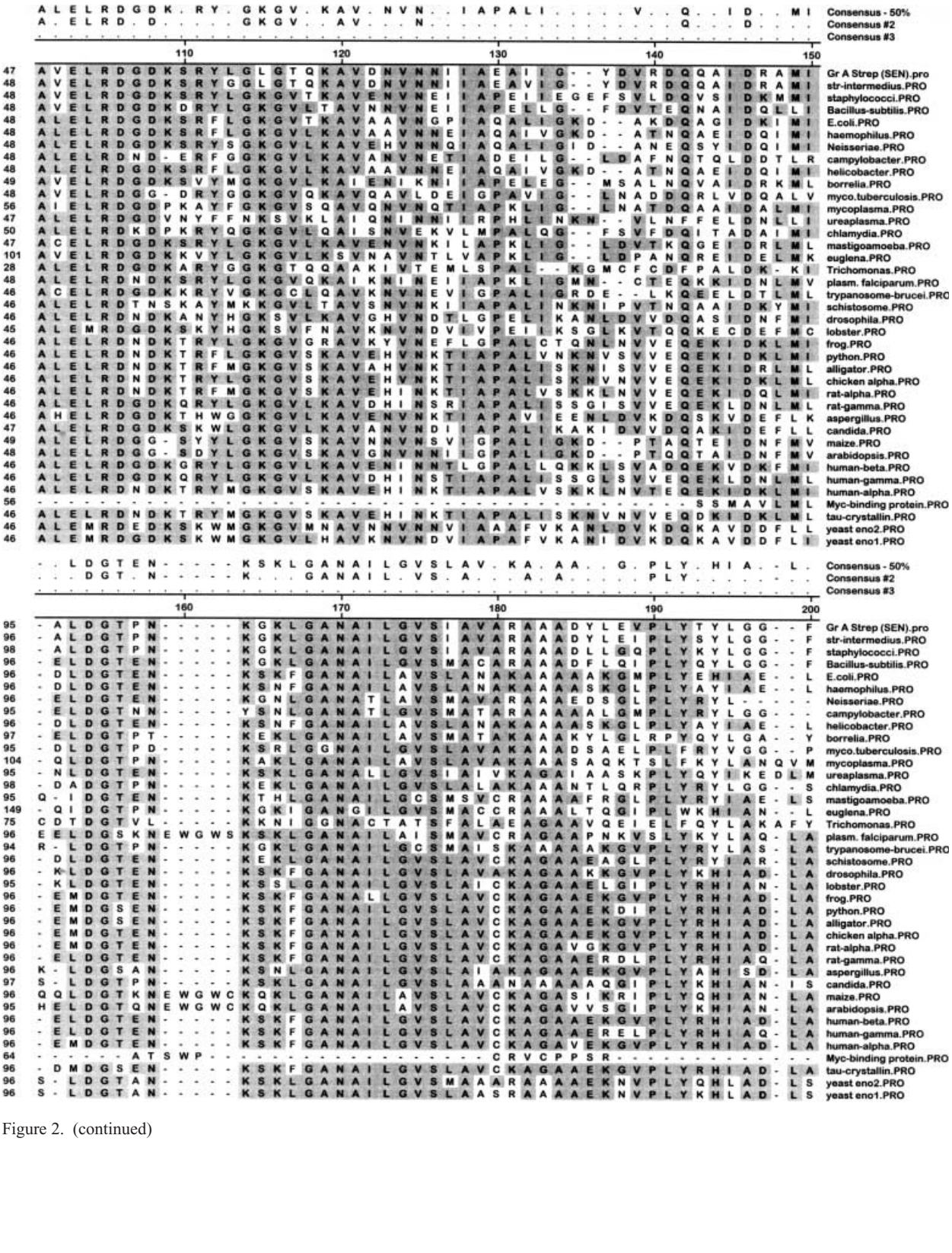


Figure 2. Comparison of amino acid sequences of enolase from different species by the Custal method using the Lasergene-MagAlign program. All sequences were obtained from Gen Bank protein sequence data with accession numbers as mentioned in brackets. Gr A strep (SEN) (Derbise A., Fischetti V. A., Pancholi V. (2000) under publication and M1 streptococcal genome sequence, http://www.genome.ou.edu/strep.html), *Strep intermedius* (BAA81815), *Staphylococci* (AAC17130), *Bacillus subtilis* (A69725), *E. coli* (AAC69289), *Haemophilus* (E64103), *Neisseriae* (AAF41661), *Campylobacter* (CAB73659), *Helicobacter* (H71967), *Borrelia* (AAC66719), *Mycobacterium tuberculosis* (B70623), *Mycoplasma* (S73562), *Ureaplasma* (AAF30591), *Chlamydia* (AAC68189), *Mastigamoeba* (AAF13454), *Euglena* (CAB96126), *Trichomonas* (AAD45336), *Plasmodium falciparum* (S42206), *Trypanosoma brucei* (AAF73201), *Schistosoma* (AAC47733), *Drosophila* (CAA34895), *lobster* (P56252), *frog* (AAC47640), *python* (AAD41646), alligator (AAA53671), chicken α (JC4186), rat α (A23126), rat γ (A24742), *Aspergillus* (23760), *Candida* (A40624), maize (S16257), arabidopsis (AAC95183), human β (NP 001967), human γ (NP 001967), human α (NP 001419), τ-crystallin (1504281A), Myc-binding protein (NP005936), yeast enolase 2 (NP 12044), yeast enolase 1 (NP 012044).



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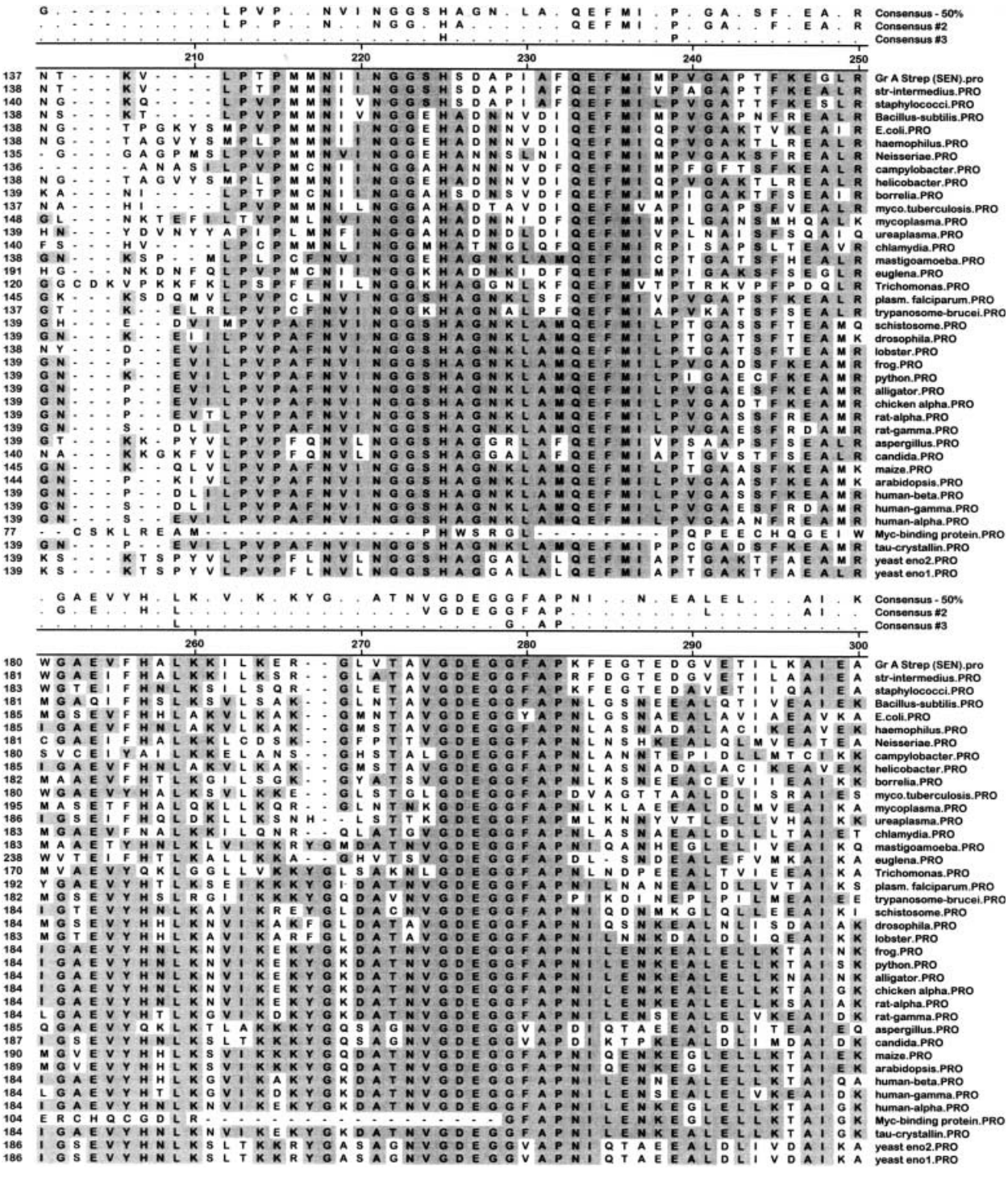

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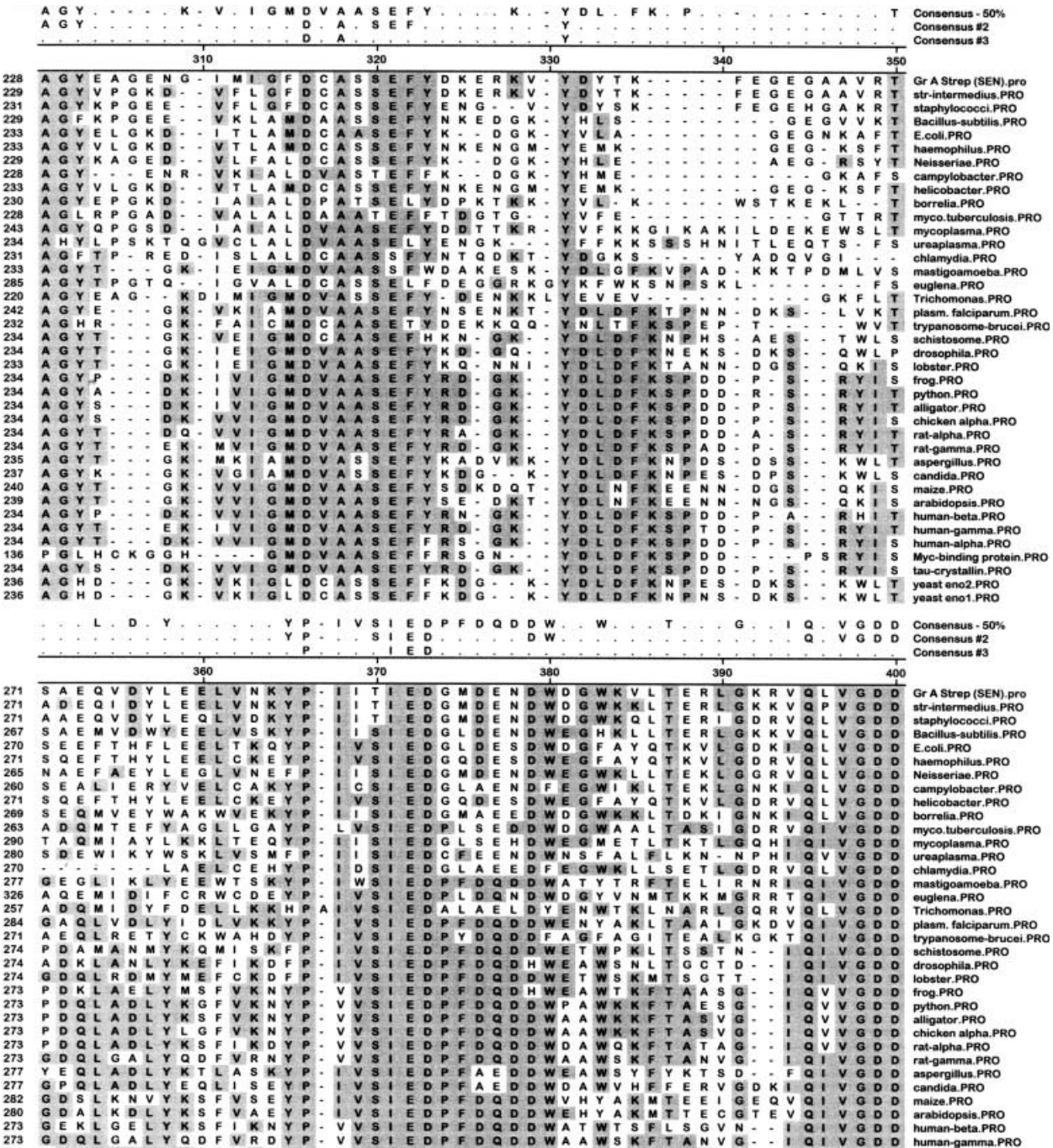

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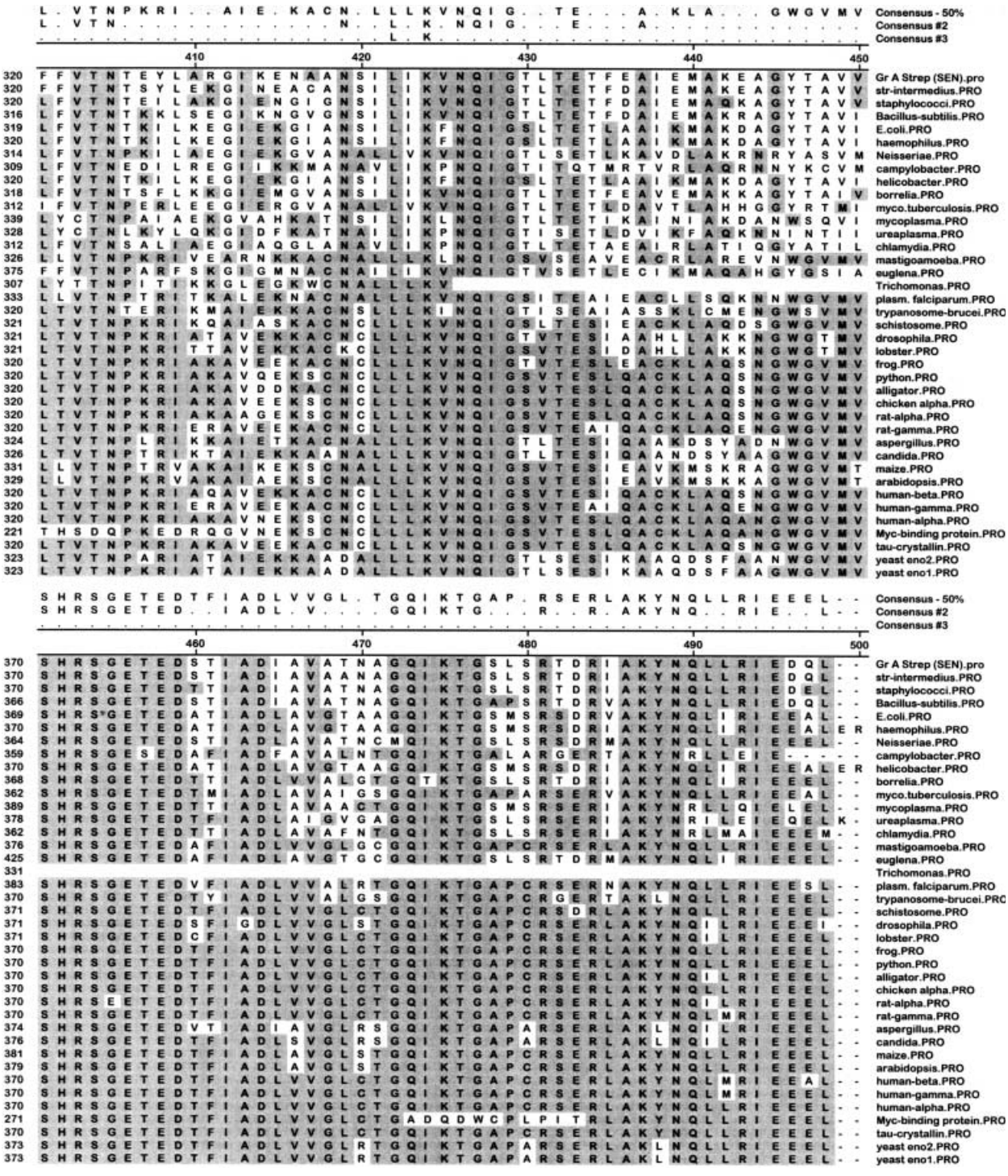

Figure 2. (continued)

Figure 2. (continued)

talytic residues also indicates that the basic folding structure of all enolases is essentially the same (figs. 2 and 5).

Crystal structure and mechanism of enolase activity

Apparently, pure crystalline enolase enzyme was first prepared from yeast by Warburg and Christian in 1941 [18]. Although earlier biochemical studies have made several important contributions in understanding the role of absolute requirements for certain divalent cations for the catalytic activity of the enolase enzyme, a deeper and more precise understanding of its mechanism of action with respect to the residues from the enolase enzyme was not available until determination of its X-ray crystal structure [24]. X-ray crystallographic data have been obtained from the crystals of yeast enolase that were grown with or without Mg^{++}, its specific substrate and metal ion/substrate inhibitors such as Zn, Mn or phosphoacetohydroxamate. In these studies, enolase crystals were grown at pH 5–7.8 using ammonium sulfate (high salt crystals), 20–28% PEG 4000 or 15–16% PEG 8000, and the corresponding X-ray data were interpreted at reasonably high resolution at 1.8–2.0 Å [20–24, 30–34]. The X-ray structure of crystalline lobster enolase has also been obtained [35]. Each study has provided interesting and important findings which are unique as well as overlapping.

On the basis of these structural investigations, several catalytic mechanisms of enolase have been proposed. In principle, enolase catalyzes the elimination of OH– from C3 of a discrete enolate intermediate which is created by removal of a proton from C2 of 2-PGA by a base in the active site in a stepwise manner (fig. 6).

Since the pK_a value for the C2 proton of 2-PGA is around 30 and that of enzymatic base is around 8–10, the major interest for all investigators lies in understanding the ability of enolase to accomplish this extremely difficult ionization step of bringing down the pK_a value.

Interpretation of kinetic data together with the several crystal structures of enolase [24–26, 30, 31, 34, 35] indicates that the groups responsible for this mechanistic process of general acid-base catalysis lie on opposite surfaces of the active site. The active site is located in a cavity at the C-terminal ends of the barrel strands (figs. 5 and 7). On the basis of site-specific mutagenesis and also from the strict conservation of residues in the active site of enolase, it is unambiguously established that mutation of any of four residues, i.e. Glu168, Glu211, Lys345 or Lys396,

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
1		90.3	79.5	70.0	56.0	55.3	60.3	50.0	55.3	58.9	53.1	51.8	40.8	54.7	46.3	51.6	37.2	47.0	46.2	42.9	42.3	42.7	42.9	43.1	43.3	43.8	42.6	43.3	44.2	46.5	45.2	45.2	43.3	43.8	42.6	23.6	42.9	45.4	45.2	1
2	9.4		79.0	70.0	56.2	56.0	60.3	50.2	56.0	59.6	55.2	52.8	42.9	54.5	46.1	51.2	38.7	45.9	45.5	42.9	41.8	42.7	41.7	43.1	42.6	43.5	42.6	43.1	44.5	46.8	45.4	45.6	42.4	43.3	42.9	23.0	42.9	44.7	44.5	2
3	20.8	22.6		72.3	56.5	55.5	61.2	50.5	55.5	59.8	54.5	51.6	42.9	53.5	45.2	52.5	36.0	46.1	45.2	45.6	44.8	45.0	44.9	45.4	45.2	46.8	45.4	44.6	45.4	44.9	45.4	44.7	45.2	46.5	44.9	45.6	24.5	45.4	45.6	46.1	3
4	35.7	37.1	31.5		61.4	62.8	66.6	55.8	62.8	61.2	56.4	53.7	44.4	56.8	49.3	52.6	38.7	50.0	47.8	45.8	44.9	45.3	45.6	45.6	45.1	46.3	45.6	46.5	46.3	47.7	47.4	47.7	44.7	46.5	45.8	23.6	45.1	45.3	45.6	4
5	58.4	59.7	57.7	49.5		83.3	61.4	52.2	83.3	57.9	52.2	54.4	45.6	50.9	46.3	52.5	39.6	44.4	47.3	46.8	48.6	45.4	48.1	48.6	49.1	50.0	48.4	47.7	45.8	49.8	47.0	48.4	50.7	47.7	47.9	26.0	49.1	46.1	46.3	5
6	56.5	56.8	56.8	48.6	15.8		63.1	51.4	99.8	55.9	53.1	51.1	45.0	51.9	46.1	51.6	39.0	43.8	46.2	45.4	44.8	45.3	45.2	45.6	45.6	46.3	46.3	45.2	44.3	45.9	45.9	47.2	46.5	45.2	45.9	24.8	45.4	42.4	43.3	6
7	51.1	52.8	50.0	42.4	50.7	46.9		56.5	63.1	58.2	55.1	54.0	45.6	55.7	50.7	53.0	39.3	49.1	46.5	47.9	47.4	45.1	48.4	47.7	48.4	49.3	48.6	48.8	45.8	48.1	46.7	47.9	48.1	48.6	48.4	25.1	48.6	46.3	46.5	7
8	73.2	72.6	69.6	57.1	68.2	65.3	57.8		51.4	51.4	52.7	48.6	44.9	51.9	46.1	47.3	35.0	45.4	45.7	42.8	43.2	43.5	44.4	43.0	43.7	44.0	42.5	43.0	43.2	45.7	43.7	44.2	43.7	43.5	43.7	24.2	43.5	44.7	44.2	8
9	56.5	56.8	56.8	48.6	15.8	0.0	46.9	65.3		55.9	53.1	51.1	45.0	51.9	46.1	51.6	39.0	47.3	46.2	45.4	44.8	45.3	45.2	45.6	45.6	46.3	46.3	45.2	44.3	45.9	45.9	47.2	46.5	45.2	45.9	24.8	45.4	42.4	43.3	9
10	54.7	54.5	52.1	51.1	55.5	59.4	54.8	68.1	59.4		51.7	55.7	44.8	54.7	47.1	54.5	39.9	47.3	46.6	45.0	43.9	43.4	44.1	44.3	44.6	44.6	43.6	45.5	43.6	45.0	46.4	45.3	43.9	46.0	43.6	23.9	44.3	41.8	41.6	10
11	66.8	62.3	61.2	59.8	66.9	63.7	61.4	69.1	63.7	68.1		49.4	41.7	51.9	45.5	48.3	37.8	46.4	46.4	45.7	44.3	44.8	45.7	45.2	45.5	46.2	45.5	47.1	46.2	48.0	48.5	47.1	46.2	47.6	45.2	27.8	45.9	45.0	46.2	11
12	70.8	69.4	69.9	67.7	64.5	69.9	65.4	75.8	69.9	64.9	74.1		49.8	53.5	44.9	47.8	40.8	45.5	44.5	45.4	44.3	44.3	43.1	43.1	43.8	43.1	42.9	43.5	43.6	44.5	43.9	44.6	42.4	44.2	43.3	22.7	43.3	43.5	44.4	12
13	94.3	86.4	85.4	83.1	81.8	82.1	82.5	82.9	82.1	83.1	90.4	73.0		43.2	39.6	43.0	30.2	39.5	38.9	43.3	40.6	40.2	42.4	41.7	42.6	41.9	42.4	41.5	39.0	38.9	40.2	40.9	42.4	41.5	41.7	23.6	42.4	38.2	38.0	13
14	65.5	66.0	65.4	59.6	69.5	66.6	60.3	64.1	66.6	66.0	67.5	66.7	85.5		45.5	47.2	37.5	46.2	45.0	45.0	43.6	43.4	45.8	45.0	44.3	45.0	44.1	45.3	43.2	43.2	44.8	44.6	45.8	45.3	44.3	23.9	45.0	41.5	42.5	14
15	78.6	79.2	79.9	71.1	77.3	78.6	67.5	79.2	78.6	75.5	79.9	82.7	93.7	80.5		51.5	37.8	62.2	62.5	62.9	61.9	62.4	63.4	62.0	63.1	63.6	61.8	65.0	55.7	57.9	61.3	59.5	64.1	65.2	63.4	35.5	63.1	55.1	55.6	15
16	64.7	67.7	63.4	65.5	66.0	66.9	63.2	76.0	66.9	60.5	72.5	73.7	84.4	79.9	65.3		35.0	44.6	48.3	45.4	46.0	46.2	46.5	45.4	45.6	46.3	45.9	47.0	43.6	46.4	48.0	48.4	45.6	47.2	46.5	28.1	46.1	42.3	42.6	16
17	97.0	97.0	102.4	92.4	93.3	94.8	92.1	99.5	94.8	92.5	94.5	93.1	131.4	95.9	97.8	105.2		40.8	39.6	36.0	36.6	36.6	37.8	36.6	35.6	36.3	35.0	36.6	40.8	41.4	35.6	36.0	37.2	37.2	35.0	12.7	36.0	38.1	38.7	17
18	70.8	73.1	70.8	62.8	78.3	78.0	65.4	75.3	78.0	69.3	70.1	75.6	92.2	75.4	45.0	75.6	82.4		58.3	59.4	59.8	62.1	63.8	63.6	64.1	64.7	62.9	65.0	60.5	58.2	65.9	66.2	63.6	65.0	63.6	37.0	64.7	57.7	59.3	18
19	77.3	77.9	76.7	72.4	75.4	78.9	73.5	78.5	78.9	73.9	73.3	82.4	97.3	78.5	49.2	75.2	91.8	53.7		56.2	57.1	55.7	57.3	55.5	57.3	57.6	56.2	59.2	56.2	57.3	56.6	53.8	59.0	59.4	56.4	31.0	56.9	54.5	55.9	19
20	82.8	81.5	77.0	74.8	74.3	74.9	71.2	87.1	74.9	74.2	73.0	76.8	85.0	75.1	43.8	74.9	94.3	49.0	58.7		71.6	72.1	71.4	71.7	72.8	72.8	71.7	70.5	61.1	62.9	63.8	64.7	71.0	70.3	74.0	42.4	73.3	61.5	62.4	20
21	83.5	84.1	78.3	75.4	70.1	77.3	71.2	83.9	77.3	77.6	75.8	80.2	91.2	78.2	45.9	76.1	92.3	48.0	56.8	35.4		77.1	74.6	71.8	73.0	72.5	72.5	70.4	63.3	65.1	64.7	64.9	71.8	71.4	72.1	40.6	72.7	63.0	64.7	21
22	83.5	82.8	77.6	76.0	75.5	76.1	77.9	82.6	76.1	81.5	76.0	84.0	91.6	81.5	46.3	76.1	95.8	45.9	60.2	34.4	26.1		71.1	70.0	71.4	71.1	69.7	71.1	60.5	62.6	65.4	66.7	70.0	71.8	70.9	42.1	70.9	61.4	62.8	22
23	80.5	82.5	77.9	74.2	71.9	74.9	68.3	81.9	74.9	76.7	72.2	82.4	86.1	73.5	43.4	73.7	89.4	39.3	55.3	34.6	30.1	34.4		89.2	89.9	91.2	86.6	82.0	60.6	61.5	65.0	66.6	83.2	82.3	87.8	50.1	89.9	60.1	61.3	23
24	79.2	77.9	76.1	73.0	68.4	71.9	69.5	85.3	71.9	75.5	73.9	81.7	88.8	74.7	45.0	76.7	91.3	39.7	59.2	34.2	34.3	37.0	11.7		93.5	94.0	89.9	83.9	62.0	61.5	65.7	66.1	82.0	83.9	90.3	51.6	92.9	60.1	61.5	24
25	79.2	79.9	77.3	74.8	67.9	71.9	68.9	83.2	71.9	74.2	73.3	80.5	86.1	76.6	43.8	76.1	94.3	38.9	55.3	32.5	32.5	34.8	10.9	6.8		95.4	91.2	85.3	63.1	63.6	65.0	66.4	83.2	85.3	91.0	51.3	94.9	60.8	62.0	25
26	77.9	77.3	73.1	72.4	66.8	70.1	66.1	82.5	70.1	73.6	70.4	81.1	87.4	75.4	43.0	74.9	92.3	37.8	54.8	32.5	33.3	35.2	9.3	6.3	4.8		91.0	85.3	62.2	64.1	66.1	67.5	83.9	85.3	92.9	52.5	97.2	61.1	62.0	26
27	81.8	80.5	77.3	74.2	70.1	70.7	67.8	85.3	70.7	75.5	73.3	81.1	86.7	77.3	45.9	75.5	97.4	41.3	57.7	34.2	33.3	37.4	14.8	10.9	9.3	9.6		82.0	62.2	62.2	64.3	65.9	82.9	82.7	94.0	52.8	90.3	59.9	61.1	27
28	79.9	79.9	78.6	71.8	72.5	73.7	68.9	86.0	73.7	72.4	69.8	79.9	88.1	75.4	40.6	74.3	90.4	37.8	52.1	36.1	36.5	35.5	20.6	18.2	16.5	16.5	20.6		61.1	63.1	64.3	65.0	82.9	98.6	82.7	47.5	84.1	59.4	61.3	28
29	77.7	75.8	75.8	73.1	76.7	80.2	73.0	80.8	80.2	80.2	70.7	80.8	94.9	81.8	57.9	80.5	81.6	48.7	58.8	50.4	47.4	54.0	50.9	47.4	45.7	47.4	47.8	50.0		72.6	55.9	55.9	62.0	61.3	61.5	34.9	62.9	70.3	71.2	29
30	75.2	73.4	68.8	70.1	69.4	75.5	72.2	78.5	75.5	74.6	66.2	81.1	94.8	81.8	54.9	72.0	82.0	53.2	56.4	47.7	42.7	48.1	49.9	49.0	46.0	45.2	48.1	47.7	30.1		57.7	58.6	62.0	62.9	62.0	37.3	63.4	75.3	77.1	30
31	77.3	76.1	74.9	69.9	71.7	73.7	71.6	84.9	73.7	74.3	67.9	78.6	88.0	77.9	45.6	68.6	98.4	40.9	55.2	40.1	38.3	38.7	37.0	35.9	37.0	35.2	38.2	38.2	54.9	50.7		87.8	64.7	64.3	66.4	40.3	65.7	54.2	54.5	31
32	76.4	75.2	74.0	70.1	70.5	72.3	69.9	84.2	72.3	73.4	72.2	77.1	86.3	75.7	47.8	68.5	95.8	39.3	59.8	38.5	38.7	37.9	33.7	34.4	34.1	32.3	34.8	36.7	54.1	49.2	12.2		65.7	65.0	67.7	41.2	66.4	52.9	54.0	32
33	81.1	82.5	76.1	76.7	65.1	70.1	69.5	82.5	70.1	76.1	72.2	83.0	86.7	74.7	42.6	77.3	92.3	40.5	52.1	35.3	34.3	37.0	19.1	20.6	19.1	18.2	19.4	19.4	48.3	49.0	37.8	35.5		83.2	83.4	49.3	83.2	61.3	62.4	33
34	78.6	79.2	78.6	71.8	71.9	73.7	68.9	84.6	73.7	71.8	68.7	78.6	88.1	74.7	40.2	73.1	88.5	37.8	51.6	36.4	35.1	34.4	20.3	18.2	16.5	16.5	19.7	1.4	49.6	48.1	38.2	36.7	19.1		83.4	47.8	84.1	59.7	61.1	34
35	81.8	79.9	77.3	73.6	71.3	71.9	68.9	81.9	71.9	76.7	73.3	80.5	88.1	77.3	43.4	74.3	97.4	40.1	57.2	30.7	34.0	35.5	13.4	10.4	9.6	7.5	6.3	19.7	49.1	49.0	34.8	31.9	18.8	18.8		57.0	92.2	61.1	62.2	35
36	163.3	161.1	153.9	151.8	152.5	149.1	149.8	159.0	149.1	149.2	136.2	153.2	155.7	152.9	94.8	139.4	184.4	91.5	114.5	82.2	86.7	81.3	62.2	56.3	57.7	55.6	57.7	66.9	107.9	103.5	84.5	79.4	67.7	66.1	48.8		53.1	35.5	36.4	36
37	80.5	79.2	76.7	75.5	69.0	72.5	67.8	83.9	72.5	74.2	71.0	80.5	86.1	75.4	43.8	75.5	94.3	37.8	56.3	31.8	32.9	35.5	10.9	7.5	5.3	2.8	10.4	17.9	46.1	46.4	36.3	34.1	19.1	17.9	8.3	54.9		60.8	61.8	37
38	76.4	77.0	74.6	71.8	77.6	83.1	73.3	79.2	83.1	82.1	72.4	82.4	97.9	83.2	58.1	82.8	90.3	52.8	60.7	51.7	48.6	50.8	53.5	53.5	52.1	51.7	53.5	55.8	35.4	28.7	55.7	58.3	51.2	55.3	51.7	104.2	52.1		95.4	38
39	76.4	77.0	72.8	71.3	77.0	79.9	73.3	79.9	79.9	82.8	70.1	80.5	99.5	80.5	57.1	82.1	86.5	49.7	57.7	49.9	45.6	48.1	51.2	50.8	49.9	49.9	51.2	52.1	33.9	26.0	55.2	55.9	49.0	52.6	49.4	100.6	50.3	4.7		39
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	

1 Gr A Strep (SEN).pro
2 str-intermedius.PRO
3 staphylococci.PRO
4 Bacillus-subtilis.PRO
5 E.coli.PRO
6 haemophilus.PRO
7 Neisseriae.PRO
8 campylobacter.PRO
9 helicobacter.PRO
10 borrelia.PRO
11 myco.tuberculosis.PRO
12 mycoplasma.PRO
13 ureaplasma.PRO
14 chlamydia.PRO
15 mastigoamoeba.PRO
16 euglena.PRO
17 Trichomonas.PRO
18 plasm. falciparum.PRO
19 trypanosome-brucei.PRO
20 schistosome.PRO
21 drosophila.PRO
22 lobster.PRO
23 frog.PRO
24 python.PRO
25 alligator.PRO
26 chicken alpha.PRO
27 rat-alpha.PRO
28 rat-gamma.PRO
29 aspergillus.PRO
30 candida.PRO
31 maize.PRO
32 arabidopsis.PRO
33 human-beta.PRO
34 human-gamma.PRO
35 human-alpha.PRO
36 Myc-binding protein.PRO
37 tau-crystallin.PRO
38 yeast eno2.PRO
39 yeast eno1.PRO

Figure 3. Percentage identitiy and divergence between the amino acid sequences of two different enolases using the Custal method.

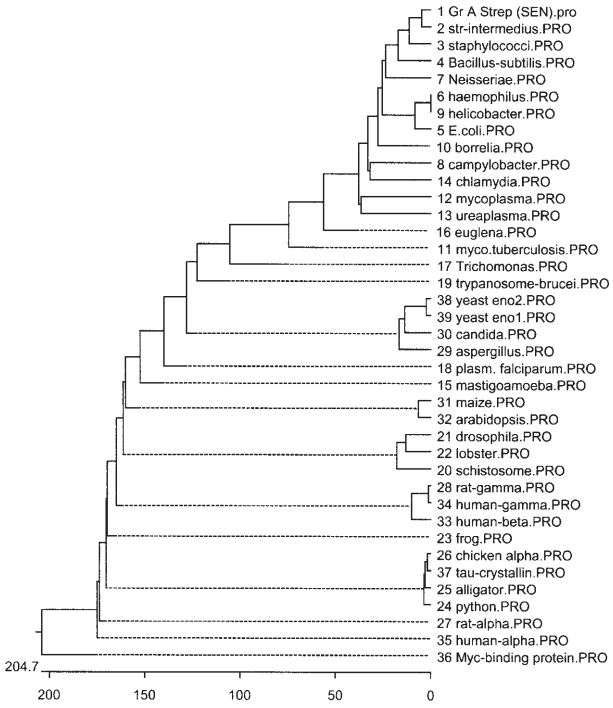

Figure 4. Phylogenetic tree of 39 enolase species using the Custal method.

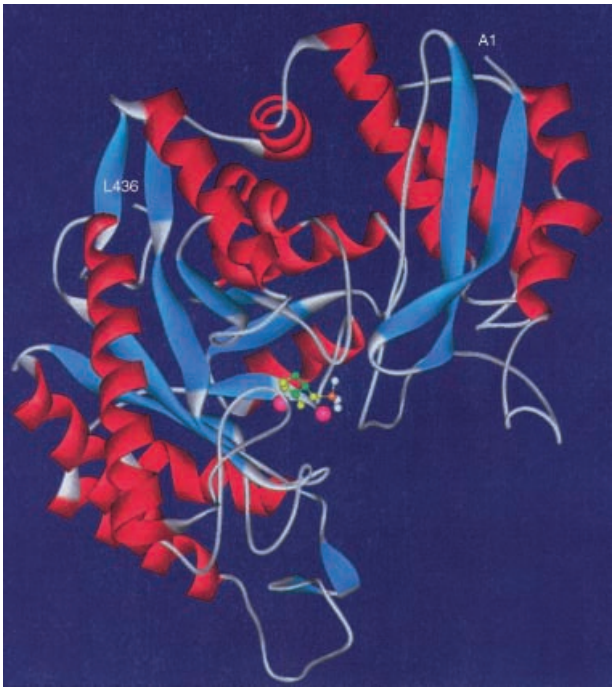

Figure 5. Crystal structure of yeast enolase: ribbon diagram of a subunit of the enolase-Mg^{2+}-2-PGA complex. The N-terminal and C-terminal ends are labeled as amino acid letter followed by residue number i.e. A1 and L436. Mg ions are represented by spheres and a ball-and-stick model of 2-PGA is shown in the active-site pocket at the C-terminal end. Data were obtained from the Brookhaven protein data bank as entry code 1ONE.pdb file/MMDB ID:5339, Larsen et al. (1995) [34]. The Figure was drawn using the WebLab ViewerLite 3.7 program.

lowers the activity in the overall reaction relative to wild-type enolase by a factor of 105. There have, however, been some ambiguity for other residues that may function as the general acid-base pair in the active site. For example, mutant His373Asn enolase retains about 10% of wild-type activity [36, 37], whereas mutant His373Phe retains only 0.0003% of wild-type enolase activity [36]. This suggests that His373 may not be essential for effective catalysis [37], but its function may involve hydrogen bonding through interaction with the Glu168/Glu211/ H_2O, which produces removal or addition of a hydroxyl at position C3 of the substrate [36]. Since His159 (His157 in lobster enolase [35]) is also located in the active site (3.0 Å away from the C2 proton of 2-PGA) and is also conserved in all species, it is also thought to play a role in the initial steps of the reaction [38]. Mutant His159Ala enolase retains 0.01% of its wild-type specific activity and fails to ionize the C2 proton of 2-PGA; hence, the role of His159 is seen as serving as a potential catalytic base in the enolase reaction [38].

Recently, finer aspects of the mechanism of enolase activity have been summarized based on X-ray crystallographic data on the crystal structure of a complex between yeast enolase and an equilibrium mixture of PGA and PEP at pH 7.8 [29, 37]. This summary is based on previous similar and complementary X-ray crystallographic studies on wild-type and mutant α-enolase [30–35] (see also figs 5 and 7).

When 2-phosphoglycerate binds to α-enolase, it interacts with Gln167, Lys396 and both essential divalent cations $[(Mg^{2+})_2]$, and as a result the carboxyl group of PGA is rotated and neutralized. A large movement of the loop Ser36-His43 (^{36}Ser-Gly-Ala-Ser-Thr-Gly-Val-His^{43}) and a smaller movement of the loops Ser158–Gly162 and Asp255–Asn256 then result into the coordination of backbone carbonyl and side-chain hydroxyl of Ser39 with catalytic Mg^{2+}. This movement appears to close the active site.

The loops, Val153–Phe169 and Ser250–Gly277, then move together, allowing His159 to donate a proton to the phosphoryl of PGA. The interaction of protonated phosphoryl of PGA with Arg374 (loop G) and catalytic Mg^{2+} then results in overneutralization of the negative charge on phosphoryl and the lowering of and hence bringing of carbon-2 pK_a of PGA into the physiological range. The movement of Lys345 (strand 6) closer to Arg374 (loop G) lowers the pK_a of its ε-ammonio group, which loses its proton and accepts one from the C2 of PGA. Finally, the proton shared by Glu168 and Glu211 forms a hydrogen bond to the PGA hydroxyl group. This proton is then transferred from the carboxylate to the hydroxyl group, making H_2O and phosphoenol pyruvate. In the reserve reaction (hydration reaction), the water held by Glu168, Glu211 and His373 is deprotonated by one of the car-

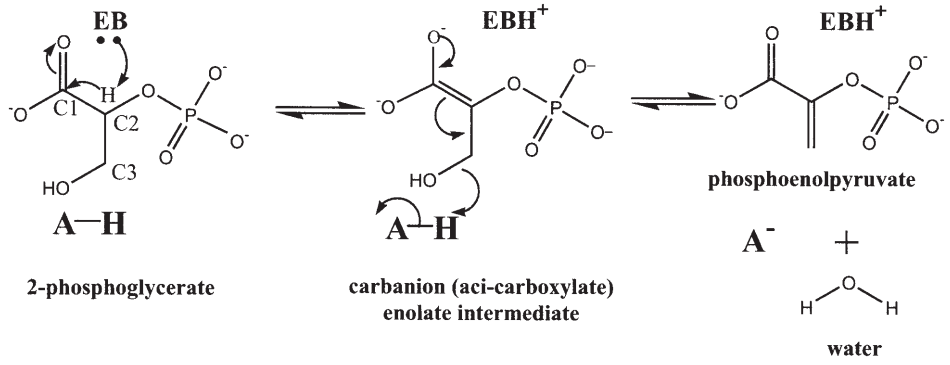

Figure 6. Stepwise model for the β-elimination reaction of enolase. A, acid-, EB, enzymic base. Formation of enolate intermediate during enolase reaction.

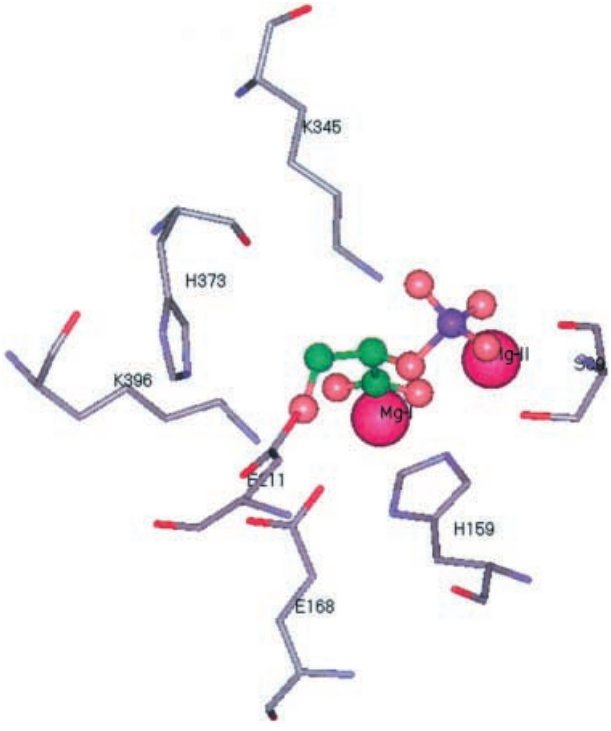

Figure 7. Active site loop yeast enolase. A ball-and-stick model representation of the active site of the enolase-Mg^{2+}-2-PGA complex. Magnesium ion and some of the surrounding side chains of catalytic residues are shown using a stick model representation. Details regarding data acquisition and figure drawing are as described for figure 5.

boxylates, and the resulting OH^- adds to C3. At the same time, a proton from the ε-ammonio group of Lys345 adds to C2, resulting in the elimination of the double bond between C2 and C3.

Enolase superfamily

Recently, on the basis of this fundamental structure and the conserved amino acid residues of the catalytic site, Babbit et al. [1] described a mechanistically diverse enolase superfamily comprising 16 enzymes which catalyze 11 different chemical reactions. The commonality of this family is that all its members catalyze the initial metal-assisted general base-catalyzed abstraction of the α proton from a carbon adjacent to a carboxylate group, resulting in a stabilized enolate anion intermediate as described above. The fate of this intermediate anion is then determined by the different active-site architecture, catalyzing a variety of chemical reactions that include racemization, cycloisomerization and β elimination of either water or ammonia using diverse carboxylate anions as substrate. Each of these enzymes contains a (β/α)-β-barrel domain. The catalytic functional groups involved in the formation of intermediate enolate anions are located in the pocket formed by the C-termini of the β strands of the barrel (fig. 5). There are three subgroups in this superfamily, which is divided according to the basic residues involved in the formation of enolic intermediates [1]: (i) an enolase subgroup which utilizes a single lysine residue, (ii) a mandelate racemase (MR) subgroup which utilizes one lysine and/or a histidine residue(s) and (iii) a muconate lactonizing enzyme (MLE) subgroup which utilizes two lysine residues [39]. Studies that recognize such a structure-function paradigm have provided a rational basis for understanding the chemistry of all of the superfamily members [1, 2].

Multifunctional nature of enolase

Since enolase is abundantly expressed in most of the cells, it has been proved useful as a model for studying basic mechanisms of enzyme action as well as structural analysis. However, unlike the gene that expresses glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the gene that expresses enolase is not a housekeeping gene since its expression varies according to the pathophysiological, metabolic or developmental conditions of cells [42]. Several recent studies have reported other seemingly unrelated functions for enolase by serendipity.

The accumulating evidence makes clear that enolase is a multifunctional protein.

Location diversity

Enolase as a surface protein

It has been uniformly reported that the expression of enolase-specific messenger RNA (mRNA) increases to a very high level in exponentially growing cells but remains almost at an undetectable level in the stationery/resting/quiescent phase [40, 41]. In yeast, expression levels of two yeast enolases are under metabolic and/or developmental control [42]. Enolase is expressed on the surface of a variety of eukaryotic cells as a strong plasminogen-binding receptor [7, 8, 43, 44]. We and others have recently shown by FACS (fluorescence-activated cell sorting) analyses that hematopoietic cells (neutrophils, B cells, T cells, monocytes) which have been stimulated with PMA (phorbolmyristateacetate) and LPS (lipopolysaccharide) express significantly higher amounts of α-enolase on their surface [45, 62]. The presence of enolase on the surface of prokaryotes was first reported in group A streptococci as a plasminogen receptor [9]. α-enolase is described as an immunodominant antigen in patients suffering from systemic candidiasis [46–49]. The presence of other glycolytic enzymes such as GAPDH and phosphoglycerate kinase on the surface of bacterial and fungal cells has also been reported [50–53]. In this context, the expression of enolase on the surface of bacteria is not unprecedented. However, a challenging question remains about how these glycolytic enzymes, including enolase, are expressed on the surface of eukaryotic cells such as hematopoetic cells, epithelial cells, neuronal cells and also on Gram-positive cocci, *Candida* and parasites. Whether mechanisms like the hydrophobic domain in (spanning 33–44 amino acids in human enolase, ^{33}AAVPSGASTGIY^{44}) or the posttranslational acylation [54] or phosphorylation [55] of enolase play a role in the membrane association and surface expression of cytoplasmic enolase are subject to further verification. However, in the absence of classical protein-sorting machinery such as signal sequences and hydrophobic domains [56, 57] and LPXTGX – a hexapeptide motif of Gram-positive bacterial surface proteins [58, 59], the expression of selective glycolytic enzymes on the microbial cell surface warrants assigning them to a special class of surface proteins. Incidentally, in almost all cases, this class of proteins belongs to organisms which possess only one outer membrane or the membrane and cell wall, but not organisms which possess two membranes, as in Gram-negative bacteria. Thus, although it remains unknown why these enzymes and, in particular, enolase appear on the surface, by virtue of its location, many new functions have been revealed.

Enolase as a surface receptor for the binding of plasminogen

Plasminogen, one of the key components of the fibrinolytic system, binds to a variety of blood cells [60, 61]. The reports by Miles and Plow [60] showed that increased plasminogen binding to thrombin-stimulated platelet cells is due to increased expression of certain integrins and the recruitment of additional fibrin which serves as the receptor for plasminogen. However, it was not known how other blood cells which do not express fibrin-binding integrins could possibly bind plasminogen with great affinity. Using the U937-monocytoid cells as a model system, the same group subsequently found that α-enolase was a candidate plasminogen receptor on nucleated blood cells [7]. Using α-enolase-specific monoclonal antibody 9C12, the researchers identified its surface-exposed epitope on neutrophils and U937 cells using the phage display method [62]. This surface-exposed epitope, which spans a stretch of 16 amino acids (spanning 257–272, ^{257}DLDFKSPDDPSRYISP^{272}), is located in the central loop of human enolase [40, 62]. It is, however, worth noting that this loop is not found in any of the reported prokaryotic enolase (fig. 2). Enolase is now known to be present on the surface of hematopoietic cells such as monocytes, T cells and B cells, neuronal cells and endothelial cells [7, 43, 44]. The expression of α-enolase on the surface of human neutrophils, T cells, B cells and monocytoid cells has been found to be dependent on the pathophysiological conditions of the these cells [45, 63]. Enolase (both $\alpha\alpha$ and $\beta\beta$ isoforms) from muscle has been shown to bind other glycolytic enzymes such as phosphoglycerate mutase, muscle creatine kinase, pyruvate kinase and muscle troponin with high affinity [16]. The high affinity of these three enzymes suggests that they may make a functional glycolytic segment in the muscle where ATP production is required for muscle contraction [16].

Nuclear location

Myc-binding protein (MBP-1), which is structurally very similar to α-enolase, has been found to be localized in the nucleus as a DNA-binding protein [64]. This aspect is dealt with more in detail in the following section.

Functional diversity

Enolase as an eye τ-crystallin protein

Crystallins are identified as the principal components of eye lens, contributing about 20–60% of total wet weight [65, 66]. The α-, β-, γ-, δ-, ε- and τ-crystallins are all components of vertebrate lens [67]. In fish, reptiles, birds and lamprey, major lens protein has been designated τ-crystallin [68]. A preliminary amino acid sequence for τ-crystallin shows strong similarity to the sequences of

human and yeast enolase (Fig. 2) [68]. τ-crystallin is present in as much as 23% of the total protein of the lens. Purified τ-crystallin shows significantly low enzymatic activity, which is equivalent to 5% of purified rabbit muscle enolase [66]. It seems that during development and differentiation, the fiber cells of the lens lose all their organelles, and hence they must rely on cytoplasmic glycolysis as a source of energy [68]. As described above, enzymatically active enolase is found in dimeric form [6, 11], and hence the low enolase activity of τ-crystallin is probably due to the fact that it is predominantly found in monomeric form as a result of the absence of an essential Mg^{++} required for dimerization. It is also believed that the loss of enzyme activity may be due to aging in the lens or the presence of some inhibitor substance in the lens [66]. Irrespective of its enzyme activity, the presence of a significant amount of enolase in eye lens is clearly indicative of its structural role in lens and cataracts.

Enolase as a Myc-binding protein (MBP-1)

C-myc is a DNA-binding phosphoprotein protooncogene. It plays a crucial role in the regulation of cell growth and differentiation [69]. The human c-myc protooncogene contains two TATA boxes separated by 165 bp located at the 5′ end of the first axon [70]. MBP-1 is a 37-kDA protein that binds in a region +123 to +153 relative to the c-myc P2 promoter [71]. MBP-1 is a negative regulator of c-myc expression and binds in the minor groove of the c-myc P2 protein simultaneously with TATA-binding protein [69, 70, 72]. Thus binding of MBP-1 to c-myc leads to tumor suppression. The sequence analysis of MBP-1 has recently shown that it has extensive homology with α-enolase [64]. Insterestingly, the start codon MBP-1 ATG corresponds to the 400 bp downstream of the α-enolase ATG, and hence MBP-1 is considered to be an alternative translation initiation product of the α-enolase RNA [64]. Since MBP-1 lacks the first 96 residues of α-enolase, it does not show enolase activity, suggesting that the N-terminal portion is essential for glycolytic activity, and is not essential for c-myc binding. However, the mutant enolase protein, which lacks amino acid residues 96–236, no longer binds to DNA, suggesting that the N-terminal portion of MBP-1 is involved in c-myc binding for the down-regulation of its expression and tumor suppression [64, 72]. Similarly, unlike enolase, which is primarily found in the cytoplasm, MBP-1 is found in the nucleus [72].

Enolase as an endothelial hypoxic stress protein

In adapting to extreme environments such as high temperature or glucose deprivation, cells often secrete or express specific proteins known as heat-shock proteins (HSPs) or glucose-regulated proteons (GRPs) [73]. Vascular endothelial cells in a similarly stressful situation such as chronic hypoxia respond by upregulating the expression of a unique set of five cell-associated stress proteins (HAPs) (M_r 34, 36, 39, 47 and 57) in a time- and oxygen concentration-dependent manner [74]. Whereas the exposure of many other cells to hypoxia induces HSP and GRP proteins, endothelial cells, in lieu of these proteins, preferentially upregulate HAP proteins [74]. The 47-kDa protein has been identified as α-enolase. It is proposed that in a hypoxic situation, upregulation of α-enolase may provide protection to cells by increasing anaerobic metabolism [75].

Enolase as an HSP

In *Saccharomyces cervesiae* several HSPs are induced at elevated temperatures. One of these HSPs, HSP48, is identified as α-enolase [76]. HSP48 is the product of the *eno1* gene and is thought to be involved in both thermal tolerance and growth control in this organism. The expression of the *eno1* gene is negatively regulated by the *HSR1* (*heat shock resistant*) gene since the HSR1 mutant renders the organism extremely resistant (≈1000-fold more resistant then the parental strain) to heat shock [77].

Role of enolase in disease

Certain nonglycolytic properties of α-enolase described above, especially those related to surface expression and plasminogen binding, indicate that enolase may play an important role in the initiation of the disease process by modulating the pericellular and intravascular fibrinolytic system. There are several examples in which enolase is also incriminated in cancer, systemic fungal disease, dental diseases and many autoimmune disorders. The role of enolase in each individual disease category is described below.

Bacterial diseases

Bacterial surface proteins play a major role in the disease process. Although enolase was described as a surface protein on many eukaryotic cells [44], Pancholi and Fischetti [9] reported, for the first time, the presence of enolase as a strong plasminogen-binding protein on the surface of bacteria with particular reference to pathogenic Gram-positive mucosal pathogens group A streptococci and pneumococci. The importance of this work lies in the fact that streptococcal surface enolase (SEN) has much stronger affinity for direct binding of plasminogen compared with other described streptococcal surface plasminogen-binding proteins (e.g. PAM protein, SDH/Plr) [50, 51, 78]. Further, the direct binding of plasminogen to intact group A streptococci using a photoactivatable cross-linker identified streptococcal enolase as the receptor for plasminogen [9]. Group A streptococci may thus bind to plasminogen preferentially via SEN and subvert the fibrinolytic activity of human plasminogen to their own advantage for tissue invasion [9]. In staphylococci, enolase has been reported as laminin (one of the extracellular

matrix proteins)-binding protein (G. H. Choi and A. J. Simpson (1998), unpublished report, amino acid sequence accession number AAC 17130), implying that it may function in the same way as extracellular matrix-binding proteins such as fibronectin-binding proteins found on the surface of Gram-positive bacteria (see [50] for related references). However, SEN, which is structurally very similar to staphylococcal enolase, does not bind to laminin, (V. Pancholi, unpublished report, fig. 2). Hence the role of enolase, if any, in laminin binding and its further implications in bacterial pathogenesis as a result of this binding is presently unknown.

Fungal diseases

Candidal enolase has been found to be present in cancerous patients affected by invasive, deep-seated candidiasis [79]. In yeast, two enolase genes have been found in tandem duplicates [80, 81]. The sequence analysis of these genes, however, does not show the presence of the C-terminal lysines (fig. 2); hence it is not known whether this invasiveness is due to its binding ability to plasminogen. Studies by Sundstrom and Aliaga [46] have also shown that enolase is an immunodominant antigen in an experimental model of *Candida* infection. Thus, the presence of free enolase or enolase-specific antibody are considered as a potentially useful markers for invasive candidiasis. Unlike Gram-positive bacteria, candidal enolase is present within the wall [82]. It is thus assumed that active enolase may also be involved in cell wall construction [82].

Fungal allergies

Enolase is identified as one of the important allergens in inhalant allergies to fungi [83]. Immunoglobulin (Ig) E antibodies specific to enolase have been found in patients allergic to *Candida albicans* [84], indicating that enolase may play an important role in hypersensitivity responses [84]. The epitope for IgE antibodies has been localized near the C-terminal end of enolase [85].

Dental disease

There is some indirect evidence which shows that enolase may have a cariogenic role. Although many studies have failed to directly demonstrate that fluoride reduces the solubility of enamel, it is proposed that inhibition of caries formation by fluoride could be due to its ability to cause an altered growth rate and hence the changes in metabolism of cariogenic microorganisms such as *Streptococcus mutans* [86]. Fluoride is known to inhibit Mg^{++}-dependent enolase in the presence of phosphate [37, 86–89]. It is proposed that this mechanism could be one of the cariostatic effects. Inhibition of enolase by fluoride may result in PEP deficiency. The latter is essential for the PEP-dependent phosphotransferase system, which is a major glucose uptake mechanism in Gram-positive bacteria [90–92]. An interesting question that need to be addressed is whether the differences in the fluoride-mediated altered growth rate of various cariogenic microorganisms correlates with the differences in the enolase sequences and its subunit structure.

Cancer and neurological disease

The cascade of protease activity initiated by the activation of plasminogen on the cell surface has significant implications on various physiological and pathological events such as wound healing, tissue remodelling, embryogenesis and the spread of transformed tumor cells [93]. It is likely that the overexpression of enolase on such cells in various cancerous conditions may result in acquiring more plasminogen and hence in the spread of tumor cells [7, 44]. Despite several reports showing the presence of an increased amount of enolase ($\alpha\alpha$, $\alpha\gamma$, $\gamma\gamma$) in the sera of patients with different cancers, its sudden appearance in the serum in the absence of any obvious cellular damage and its direct role, if any, in the causation of cancer is not clear.

Although the role of enolase by virtue of its ability to bind plasminogen can possibly be viewed in tumor formation and metastasis [7], enolase has been considered to be a diagnostic marker for many tumors (see review [94]). Many reports are available demonstrating the direct correlation between increased expression of enolase both at the DNA level and at the protein level, and progression of tumors, such as neuroendocrine tumors, neuroblastoma and lung cancers, including small-cell carcinoma [13, 14, 95–99]. In fact an increase in serum levels of neuron-specific enolase (NSE, $\alpha\gamma$ and $\gamma\gamma$ isoforms) is considered to be a prognostic marker for hypoxic brain injury after cardiac arrest [100]. NSE in serum can also indicate Alzheimer's and psychiatric disorders or ischemic injury [101, 102]. Since NSE is also seen in a serum of lung cancer patients, as described above, or melanoma [103], it is no longer considered just a specific marker of nervous system damage. NSE is especially useful for monitoring the extent of disease and response to treatment in patients with advanced cancer, for whom intrusive procedures are inadvisable [98, 104].

Autoimmunity

Anti-enolase antibodies have been incriminated in a variety of autoimmune diseases (table 1). One of the first reports on the implication of anti-enolase antibody in autoimmune systemic rheumatic diseases was provides by Rattner et al. [105], who found 48-kDa protein (enolase)-reacting autoantibodies in sera from patients that reacted with centrosomes. Centrosomes or centrioles are the microtubule organizing focal point of the cell that play a key role in organizing the interphase cytoskeleton. This organization seems to be affected by heat-shock-related stresses [106]. Enolase is identified as an HSP [76]. Although enolase may not have a specific role at the cen-

Table 1. Enolase and its reported functions.

Functional diversity	
• τ-Crystallin	
• Plasminogen binding receptor	
• Myc-binding protein	
• Hypoxic stress protein	
• Heat-shock protein	
• Site-specific organization of tubule/centrosome	
Role in microbial diseases	
• Bacterial diseases	Plasminogen-binding receptor in group A streptococci/pneumococci
• Fungal diseases	*Candda albicans* immunodominant antigen, invasive candidiasis
• Fungal allergies	*Candida albicans* and *Aspergillus* enolase-specific IgE responses
• Dental diseases	*Strep. intermedius, Strep. mutans* – mediated dental caries (??)
• Cancer/neurological	Tumor formation/metastasis (through plasminogen binding)/tumor marker
Autoimmunity	
• Anti-centrosome antibody	
• Anti-neutrophil cytoplasmic antibodies (ANCAs)	
• Vasculitis	
• Systemic lupus erythematosus	
• Nephritis	
• Ulcerative colitis	
• Crohn's disease	
• Primary sclerosing cholangitis	
• Biliary cirrhosis	
• Autoimmune hepatitis	
• Polyglandular candidal ectodermal dystrophy	
• Discoid lupus erythematosus	
• Cancer-associated retinopathy (apoptosis)	
• Endometriosis	
• Acute rheumatic fever	
• Poststreptococcal neurological disorder/obsessive compulsive disorder/Tourette's syndrome (?)	

trosome, its presence may have a direct or indirect role in the site-specific organization of tubules [106]. The presence of anti-enolase antibodies and their binding to centrosomes may affect critical cellular functions.

A similar example of an ability of enolase to bind other glycolytic enzymes and to remain in a complex form with other cytoplasmic proteins of neutrophils has also been implicated in autoimmune diseases [107]. Moodie et al. [107] reported the reactivity of anti-neutrophil cytoplasmic antibodies (ANCAs) to cytoplasmic enolase in 37.3% of clinically proven vasculitis patients. The presence of ANCAs has also been seen in other autoimmune disorders such as systemic lupus erythematosus. They also observed the presence of enolase-reactive ANCAs in 10/41 SLE sera; 80% of these positive patients had active nephritis [107]. A recent study by Pretsi et al. [108] also demonstrated nephritis in 66.7% of SLE patients showing the presence of enolase reactive ANC antibodies. These two studies strongly indicated that anti-enolase antibodies are associated with active renal disease in patients with SLE. It is hypothesized that the high level of enolase content of the kidney and the fact that several cell types, including endothelial cells, express enolase on the membrane or cell surface [7, 8, 43, 44], autoantibodies reactive with such antigens could easily form local immune complexes. This, in turn, may play a significant role in renal and endothelial injury [108].

Anti-neutrophil cytoplasmic antibodies have also been found to contain enolase reactive antibodies in about 10% of patients with ulcerative colitis, an 18% of patients with Crohn's disease [109]. Similarly, enolase reactive ANCAs are also found in 27% of patients (4 out of 15) with primary sclerosing cholangitis [100], about 28–30% of patients with primary biliary cirrhosis [111] and 31–60% of patients with autoimmune hepatitis [111]. Once again, the clinical or pathophysiological role of these autoantibodies in inflammatory bowel or liver disease is not completely understood.

Subsequent to the findings showing the reactivity of ANCAs with neutrophilic enolase, a report by Peterson et al. [112] demonstrated a strong association of anti-enolase antibodies in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (polyglandular syndrome type 1 [APS1]). This study analyzed sera from 44 APS-1 patients and showed the highest antibody reactivity with enolase (33 positive of 41 tested, 80%) followed by HSP90 (27 of 40 tested, 67%), alcohol dehydrogenase (28/44, 64%) and pyruvate kinase (25/40, 62,5%). Although role of anti-enolase antibodies in this autoimmune disease is not clear, the authors consistently observed the absence of *Candida* anti-HSP90 antibody which is present in superficial mucocutaneous infection and has been shown to have a role in the prevention of systemic spread of infection [112]. In light of this finding, the functional relation of these two proteins and corresponding antibodies in modulating candidal infection and its association with APS1 is worth exploring.

While the above study [112] corroborates the findings described by Walsh et al. [79], in terms of the role of anti-enolase antibodies in systemic candidal infections and autoimmune disease, Gitlis et al. [113] showed the role of high titres of anti-enolase autoantibodies also in a patient with discoid lupus erythematosus, which is a cutaneous form of lupus erythematosus that rarely disseminates to SLE. Unfortunately, this report showing the IgG1 autoantibodies to evolutionarily conserved autoepitopes of enolase was based on the serum of only one patient with noninvasive, local cutaneous lesions. Hence, the role of anti-enolase autoantibodies in local, nonsystemic and noninvasive disease may require further confirmation to stipulate their broad association.

The association of enolase autoantibodies has also been seen in patients with CAR (cancer-associated retinopathy) syndrome [114]. CAR is an uncommon paraneoplastic disease in which degeneration of the retina occurs as a result of several types of cancers (lung, bladder,

prostate, salivary gland, gastrointestinal-tract cancer, chronic lymphocytic leukemia). This paraneoplastic retinopathy syndrome includes retinal degeneration and systemic tumor outside the eye, resulting in blurred vision, night blindness, impaired color vision and complete loss of vision. In fact, this was the first report which showed the presence of serum antibodies to retinal enolase in patients with cancer. Sincer anti-enolase autoantibody was also seen in a small percentage of healthy individuals without evident tumor or retinopathic symptoms, the same group examined the specificity of the high titers of anti-enolase antibody and defined the epitopes of enolase that contributed to this heightened immune response [115]. Their study revealed four major epitopes of the enolase that bind strongly to the sera from CAR patients. Residues 31–38 (^{31}FRAAVPSG^{38}), 176–183 (^{176}ANFREAMR^{183}), 421–428 (^{421}AKFAGRNF^{428}) and 56–63 (^{56}RYMGKGVS^{63}) were found to be common for all autoantibodies, with the last epitopes associated with pathogenic sera only [115]. More interstingly, sera containing anti-enolase autoantibodies from patients with CAR syndromes are able to induce apoptotic cell death of E1A.NR3 retinal cells, which indirectly suggests the mechanism of retinal degeneration [115].

It has been suggested that an autoimmune reaction may be one of the major causes of endometriosis [116]. Despite several attempts to establish the etiology of endometriosis, which is characterized by pelvic pain, menstrual disorders, and inflammatory and immunologic changes resulting in infertility, the nature of the possible antigen remains unclear. While attempting to find the disease-specific autoantibodies generated against the cellular components of endometrial cells as a potential serological marker of endometriosis, Walter et al. [117] identified enolase-specific autoreactive antibodies in the sera from patients with endometriosis. They also localized two linear regions of 35 (aa 53–87) and 32 (aa 207–238) amino acids within the sequence of enolase that were significantly recognized by sera from endometriosis patients. However, most of these sera reacted with the former epitope that spans residues 53–87 of the enolase molecule. As noted before, it is the same stretch of amino acid sequence that is also recognized by the sera obtained from CAR patients (aa 56–63) [115]. It seems from these studies that this epitope of the enolase molecule may be a predominant autoantigenic epitope.

The role of enolase in poststreptococcal rheumatic heart disease has also been recently investigated [45]. This study evaluated antibody titer against streptococcal enolase and human enolase in the sera of rheumatic fever patients and compared the anti-enolase antibody titers in sera obtained from patients with uncomplicated streptococcal pharyngitis and healthy controls in groups of adults and children. Evaluation of antibody titers clearly indicated higher levels of enolase-reacting autoantibodies in patients with acute rheumatic fever compared with that in pharyngitis or control groups. The relevance of streptococcal enolase as a cross-reactive antigen was emphasized in this study because of the fact that the same sera did not show any increase in titer against another streptococcal glycolytic enzyme streptococcal surface GAPDH (streptococcal surface dehydrogenase, SDH) [50] or other streptococcal antigens as seen in another study using the same batch of ARF sera [118]. Since the enolase is known to be expressed on the surface of brain cells [8], it is reasonable to expect anti-enolase (SEN) circulating autoantibodies may play an important role in the development of poststreptococcal neurological disorders such as chorea [9, 45]. In light of interesting findings by Allen et al. [119] who showed elimination of circulating antineuronal antibodies by plasma pheresis in patients with obsessive-compulsive disorders improved the symptoms of the disease, it is likely, although not proven, that anti-enolase antibodies may play an important role in many neuropsychiatric disorders such as obsessive-compulsive disorder and Tourette's syndrome, and other neurological disorders.

It is obvious from the accumulating evidence that α-enolase is an important glycolytic enzyme which may perform a variety of important cellular functions in prokaryotes and eukaryotes. However, some of the most interesting and challenging issues that need to be addressed are (i) to discern the mechanism of its export to the surface, (ii) the precise role of enolase in microbial pathogenesis and (iii) pathophysiological processes, including autoimmune diseases.

Acknowledgement. V. P. is an Established Investigator from American Heart Association. The author gratefully acknowledges research grant support from the American Heart Association and the National Institute of Health (AI 42827 to V. P.). The author also thanks Drs Vincent A. Fischetti, Emil Gotschlich and John B. Zabriskie (Rockefeller University) for their encouragement, support and interest in this subject.

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