A class I (Schiff base) fructose-1,6-bisphosphate aldolase of halophilic archaebacterial origin

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The presence of a salt-dependent class I type (schiff base-forming and no metal requirement) fructose bisphosphate aldolase activity is shown for the first time in a halophilic archaebacterium.

Archaebacteria *Halobacteria* *Halophilic class I aldolase*

1. INTRODUCTION

The enzyme fructose-1,6-bisphosphate (FDP) aldolase (EC 4.1.2.13), shows properties of the class I type (Schiff base-forming, no metal requirement) in animals, plants and green algae [1]. Most lower forms of life contain a class II (metal-requiring) aldolase, although certain exceptions to the notion that bacterial aldolases belong to class II are known [2]. We had demonstrated previously that the aldolase activity in *Halobacterium halobium* was typical of a bacterial class II aldolase [3]. Therefore, it was quite unexpected that the properties of aldolase in another extreme halophile, viz. *Halobacterium* R-113, with respect to the mechanism of action, were totally distinct from those described for *H. halobium*. They also differed from those of most bacterial aldolases [1,2] in being of the class I type. As yet no class I aldolase has been reported from archaebacteria. This paper describes the properties of halobacterial class I aldolase.

2. MATERIALS AND METHODS

Halobacterium strain R-113 was a gift from Dr H. Kawasaki, Department of Agricultural Chemistry, Sakai, Japan. It was grown in high salt (25% NaCl) medium as described by Kawasaki et al. [4]. Other conditions for growth, harvesting and preparation of crude cell-free extracts were according to [3]. Halophilic aldolase activity in crude extracts was assayed in 3 M KCl/50 mM Tris buffer (pH 8.0) by using a modification of the colorimetric method of Sibley and Lehninger [3,5]. Protein concentration was determined by the method of Lowry et al. [6].

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3. RESULTS AND DISCUSSION

3.1. *Halophilic nature of aldolase activity*

Aldolase activity in cell-free extracts of *Halobacterium* R-113 was assayed in the presence of varying concentrations of salts as shown in fig.1. The enzyme activity was enhanced by increasing concentrations of KCl and CsCl, reaching a maximum around 3 M. The enzyme thus resembles *H. halobium* aldolase as well as many other enzymes from halobacteria in being a halophilic enzyme [7]. The activity was inhibited by NH_4Cl and LiCl. Unlike *H. halobium* aldolase, considerable activity could be detected at low salt concentration (0.5 M), and NaCl caused no particular enhancement.

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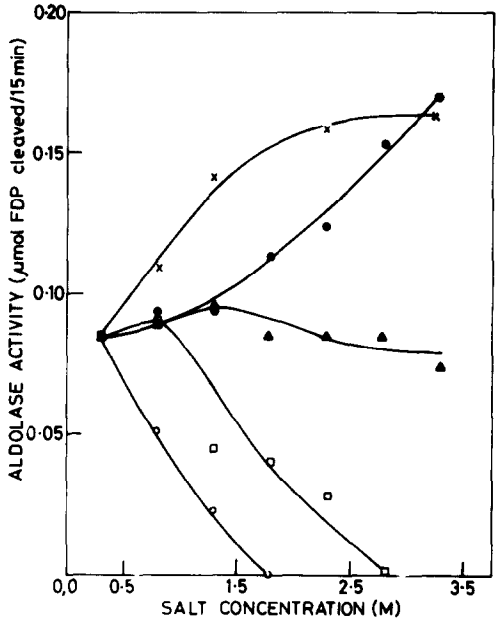

Fig.1. Effect of salts on FDP aldolase activity from *Halobacterium* R-113. Aldolase activity was assayed at 37°C by the method of D'Souza and Altekar [3]. Salts as chlorides were added to 2-ml reaction mixtures containing 100 μmol Tris-HCl (pH 8.0), 10 μmol FDP, 70 μmol hydrazine sulfate and 3.8 mg protein. Values for salt concentration indicate the final concentration in the reaction mixture. Reaction was terminated with 2 ml of 10% trichloroacetic acid after 30 min. Triose chromogens were developed in 1 ml trichloroacetic acid supernatant, read at 540 nm and corrected for appropriate blanks. Aldolase activity is expressed as μmol FDP cleaved/15 min at 37°C: (●) KCl, (▲) NaCl, (□) LiCl, (×) CsCl, (○) NH_4Cl.

3.2. *pH requirement*

The pH profile for FDP cleavage activity showed similar activities over the pH range 7.2–9.0 in Tris-HCl buffers (not shown). *H. halobium* aldolase activity was found to be optimum at pH 7.0 [3]. Mammalian aldolases are known to have a broad pH range for activity [1].

3.3. *Non-requirement for metal ions*

The extract showed aldolase activity in the absence of added divalent metals. Initial tests in which divalent metal chlorides of Mg^{2+}, Fe^{2+}, Ca^{2+} and Zn^{2+} (5 mM) were added separately to the assay mixture showed no significant enhancement in activity (not shown). There was also no inhibition in the presence of 100 mM EDTA, suggesting that a divalent metal was not required for aldolase activity. The possibility of a metal being bound tightly to the enzyme was investigated (table 1). The extract was preincubated at a high concentration of EDTA, excess EDTA removed by dialysis and the activity determined on addition of metal ions. Following the presumable removal of any bound metal by this treatment, the activity was neither lost nor affected by the addition of metal ions. The non-requirement for a metal was confirmed by determining the activity in the presence of *o*-phenanthroline, α,α'-bipyridyl and pyrophosphate (1 mM): these metal chelators were not inhibitory (table 1). These results are in total contrast to the metal dependency of *H. halobium* aldolase activity demonstrated in [3].

3.4. *Inactivation due to $NaBH_4$ treatment*

The FDP aldolases which are mainly found in the lower forms of life such as bacteria, fungi and yeasts are inhibited by EDTA and are therefore metalloenzymes, designated as class II aldolases [1]. In contrast, those termed class I aldolases and present mostly in animals, plants and green algae form a Schiff base intermediate with the substrate [8]. This intermediate undergoes reduction in the presence of borohydride, resulting in irreversible inactivation of the enzyme. Since neither a dependence on a metal cofactor nor inhibition by EDTA could be demonstrated for aldolase activity in *Halobacterium* R-113, it does not belong to class II. Furthermore, the aldolase activity could be demonstrated to be of the Schiff base-forming type, as is characteristic for class I aldolases (table 2). The enzyme activity was lost to an extent of 65% when borohydride treatment was carried out in the presence of two substrates, viz. FDP or dihydroxyacetone phosphate (DHAP).

3.5. *Sulfhydryl requirement*

We also determined the effects of heavy metals and sulfhydryl reagents or compounds on the activity of *Halobacterium* R-113 aldolase under the same conditions as in table 1, except the reaction mixture contained 6.0 mg protein and additions of

Table 1

Effect of divalent metals and metal chelators on aldolase activity in *Halobacterium* R-113

Conditions	Aldolase activity	% activity
(a)		
Untreated extract	0.130	100
EDTA-treated extract	0.119	92
Additions to dialysed extract		
None	0.129	99
Mg^{2+}	0.083	64
Ca^{2+}	0.096	74
Fe^{2+}	0.088	68
Co^{2+}	0.054	42
(b)		
Metal chelators		
EDTA	0.199	92
α,α'-Bipyridyl	0.123	95
Pyrophosphate	0.199	92
o-Phenanthroline	0.077	59

(a) Cell extract was preincubated with 100 μmol EDTA for 60 min at 4°C in 3 M KCl/50 mM Tris-HCl (pH 8.0). The extract was next dialysed in the cold against the same buffer with a change of buffer every 30 min for 2 h. Aliquots of 100 μl containing 3.5 mg protein were assayed for activity in 3 M KCl, Tris buffer (pH 8.0) in the presence of 3 μmol metal chlorides or $FeSO_4$. (b) Inhibitors (2 μmol), as indicated, were added to untreated extract. Other conditions as in fig.1. Aldolase activity is expressed as μmol FDP cleaved/15 min

Table 2

Effect of $NaBH_4$ on *Halobacterium* R-113 aldolase activity in the presence of substrates

Treatment	*Halobacterium* R-113 aldolase		Rabbit muscle aldolase	
	Activity	% activity	Activity	% activity
FDP	0.243	100	0.130	100
$NaBH_4$	0.232	96	0.136	105
$NaBH_4$ + FDP	0.085	35	0.045	35
DHAP	0.254	105	0.158	116
DHAP + $NaBH_4$	0.085	35	0.011	8

200 μl cell extract (7.0 mg protein) in 200 μl of 2 M Tris-acetate buffer, pH 6.2, containing 10 μmol FDP or DHAP was reduced by the addition of 10 μl each of 1 M $NaBH_4$ and 0.5 M acetic acid. The reduction was repeated 4 times at 3-min intervals. Residual aldolase activity was determined as in table 1. Rabbit muscle aldolase was treated similarly but in the absence of KCl. Aldolase activity is expressed as μmol FDP cleaved/15 min

5 μmol were made (control activity 0.204 μmol FDP cleaved/15 min at 37°C). 2-Mercaptoethanol and cysteine enhanced the enzyme activity (335 and 125%, respectively) whereas Cu^{2+} (22%), Hg^{2+} (15%), *N*-ethylmaleimide (86%) and iodoacetamide (30%) were partially inhibitory.

Since class I aldolases are mostly found in higher forms of life, their occurrence in microorganisms is considered to be unusual. Moreover, a Schiff base-forming aldolase has yet to be reported in halobacteria, now assigned to a separate group designated archaebacteria [9]. Some of the documented examples of eubacterial class I FDP aldolases have been found in *Peptococcus aerogenes* [10], *Lactobacillus casei* [11], *Escherichia coli* under specific conditions [12], and staphylococci [13]. The above-described results show that the *Halobacterium* R-113 halophilic aldolase can be added to the small family of microbial class I FDP aldolases. The purification of this unusual halobacterial aldolase for comparison with other class I aldolases as well as its evolutionary significance in archaebacteria is under investigation.

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