Iron-sulfur cluster-containing L-serine dehydratase from Peptostreptococcus asaccharolyticus: correlation of the cluster type with enzymatic activity.

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Iron–sulfur cluster-containing L-serine dehydratase from *Peptostreptococcus asaccharolyticus*: correlation of the cluster type with enzymatic activity

A.E.M. Hofmeister, S.P.J. Albracht, W. Buckel

Abstract Investigations were performed with regard to the function of the iron–sulfur cluster of L-serine dehydratase from *Peptostreptococcus asaccharolyticus*, an enzyme which is novel in the class of deaminating hydro-lyases in that it lacks pyridoxal-5'-phosphate. Anaerobically purified L-serine dehydratase from *P. asaccharolyticus* revealed EPR spectra characteristic of a [3Fe-4S]⁺ cluster constituting 1% of the total enzyme concentration. Upon incubation of the enzyme under air the intensity of the [3Fe-4S]⁺ signal increased correlating with the loss of enzymatic activity. Addition of L-serine prevented this. Hence, active L-serine dehydratase probably contains a diamagnetic [4Fe-4S]²⁺ cluster which is converted by oxidation and loss of one iron ion to a paramagnetic [3Fe-4S]⁺ cluster, resulting in inactivation of the enzyme. In analogy to the mechanism elucidated for aconitase, it is proposed that L-serine is coordinated via its hydroxyl and carboxyl groups to the labile iron atom of the [4Fe-4S]²⁺ cluster.

Key words: L-Serine dehydratase; Electron paramagnetic resonance; [3Fe-4S]⁺ cluster; [4Fe-4S]²⁺ cluster; Non-redox iron-sulfur protein; *Peptostreptococcus asaccharolyticus*

1. Introduction

L-Serine dehydratases and L-threonine dehydratases catalyze the irreversible deaminations of L-serine and L-threonine to pyruvate and 2-oxobutyrate, respectively. These deaminations proceed via an initial dehydration. Therefore, L-serine dehydratases and L-threonine dehydratases both belong to the systematic sub-class of hydro-lyases (EC 4.2.1). The overall reactions consist of three partial reactions: (1) dehydration of the β-hydroxamino acid to the enamine, (2) tautomerization of the pyridoxal-5'-phosphate (PLP) as a prosthetic group. During dehydration of the enamine to the oximine and (3) hydrolysis of the oximine to the product, (1) confirm the presence of a [3Fe-4S]⁺ cluster in the inactive enzyme, (2) suggest that the [3Fe-4S]⁺ cluster originated from an EPR silent [4Fe-4S]²⁺ cluster of the active enzyme by oxidation and loss of one iron atom, and (3) suggest that this [4Fe-4S]²⁺ cluster is involved in substrate binding and in catalysis by facilitating the elimination of the hydroxyl group of L-serine.

2. Materials and methods

L-Serine dehydratase was routinely purified anaerobically from 100 g wet cells of *P. asaccharolyticus* as reported in [2]. Enzyme fractions obtained after molecular sieving were apparently homogeneous as judged by SDS-PAGE. These fractions were pooled and concentrated with Centricon 30 microconcentrators. L-Serine dehydratase was assayed spectrophotometrically in a coupled reaction with lactate dehydrogenase and NADH [2].

EPR measurements at X-band (9 GHz) were obtained with a Bruker ECS 106 EPR spectrometer at a field modulation frequency of 100 kHz. Cooling of the sample was performed with an Oxford Instruments ESR 900 cryostat with an ITC4 temperature controller. L-Serine dehydratase (0.8 mg/ml or 3.5 mg/ml) in a total volume of 300 μl in quartz tubes was frozen anaerobically in liquid nitrogen. Incubations with L-serine, K₄[Fe(CN)₆] or sodium dithionite were performed at room temperature and samples were subsequently frozen in liquid nitrogen. Photoreduction was carried out according to [3] in 50 mM Tricine/KOH, pH 8.0, with 10 μM Coenzyme F₄20 in the presence of EDTA by illumination for 15 min. Spin concentrations were determined as described in [4].

3. Results and discussion

Anaerobically purified L-serine dehydratase exhibited EPR signals with line shapes which were dependent on the buffer. In 50 mM potassium phosphate, pH 7.5, the enzyme showed a rhombic signal with g-values at 2.001, 2.014 and 2.028 (Fig. 1, trace A), while a more axial signal around g = 2.01 was observed in 50 mM Tricine/KOH, pH 8.0 (Fig. 1, trace B). A rhombic signal with a third line shape was detected in 50 mM HEPES, pH 7.5, the g-values of which could not readily be determined. The EPR signals disappeared above 30 K due to

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Fig. 1. EPR spectra of L-serine dehydratase from P. asaccharolyticus in different buffers. (A) L-serine dehydratase (0.8 mg/ml) in 50 mM potassium phosphate, pH 7.5 and (B) L-serine dehydratase (3.5 mg/ml) in 50 mM Tricine/KOH, pH 8.0. EPR conditions: temperature, 12K; microwave frequency, 9419 MHz; microwave power, 2.6 mW; modulation amplitude, 0.638 mT.

Fig. 2. EPR spectra of L-serine dehydratase from P. asaccharolyticus in different buffers after incubation with L-serine. (A) L-serine dehydratase (0.8 mg/ml) in 50 mM potassium phosphate, pH 7.5, and (B) L-serine dehydratase (3.5 mg/ml) in 50 mM Tricine/KOH, pH 8.0, each after incubation with 50 mM L-serine. EPR conditions as in Fig. 1.

Table 1

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Spin concentration (% of enzyme concentration)</th>
<th>Loss of activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>0.4</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>1.0</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td>24.0</td>
<td>96</td>
<td>100</td>
</tr>
</tbody>
</table>

L-Serine dehydratase (3.5 mg/ml) in 50 mM Tricine/KOH, pH 8.0 was incubated under air. At the times indicated samples were withdrawn for activity measurements and EPR spectra were recorded for determination of the spin concentrations. EPR conditions as in Fig. 1.
tion with the substrate. In analogy to the mechanism elucidated for aconitase [9], the labile iron atom of the [4Fe–4S]^{2+} cluster could be coordinated to the β-hydroxyl group and to the carboxyl group of L-serine. This iron atom would thus act as a Lewis acid and facilitate elimination of the hydroxyl group, an otherwise poor leaving group. Elimination of water would then lead to a free enamine which would subsequently tautomerize and hydrolyze to pyruvate and ammonia in a non-enzymatic reaction (Fig. 3). Stereochemical evidence supports this mechanism [10]. In contrast, in the mechanism established for pyridoxal-5′-phosphate-dependent L-threonine dehydratases the -proton and not the β-hydroxyl group is primarily activated. The methyl group of L-threonine facilitates the removal of the hydroxyl group by stabilizing the resulting secondary carbocation at C-3, thus making the activation unnecessary [11].

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References