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

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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-hydroxyamino acid to the enamine, (2) tautomerization of the enamine to the oximine and (3) hydrolysis of the oximine to the 2-oxo acid and ammonia.

Most L-threonine dehydratases have been shown to contain pyridoxal-5′-phosphate (PLP) as a prosthetic group. During catalysis a Schiff base is formed between PLP and the β-hydroxyamino acid. As a result, the α-proton is activated and can be removed either prior to or simultaneously with the β-hydroxyl group, yielding the enamine intermediate. The following tautomerization to the imine of 2-oxobutyrate also proceeds while bound to PLP. The final hydrolysis regenerates PLP and releases ammonia and 2-oxobutyrate [1].

In contrast to L-threonine dehydratases, none of the bacterial L-serine dehydratases investigated to date has been conclusively proven to be dependent on PLP [1]. An L-serine dehydratase unequivocally devoid of PLP has been discovered in *Peptostreptococcus asaccharolyticus* [2]. The enzyme was inactivated by exposure to air and could be specifically reactivated by incubation with Fe^{2+} under anaerobic conditions. L-Serine dehydratase from *P. asaccharolyticus* was shown to contain stoichiometric amounts of non-heme iron and acid-labile sulfur, sufficient to form one [4Fe–4S] cluster per heterodimer. Here we report on EPR spectroscopical investigations which (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, K$_3$[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 F$_{420}$ 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
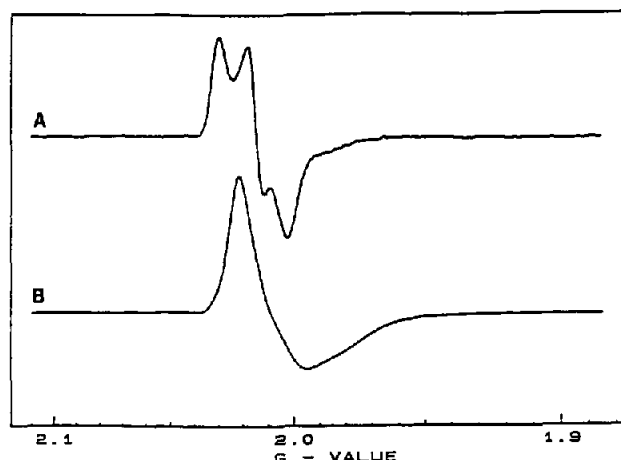
Fig. 1. EPR spectra of L-serine dehydratase from *P. asaccharolyticus* in different buffers. (A) L-Serine dehydratase (0.8 mg/ml) in 50 mM potassium phosphate, pH 7.5 and (B) L-serine dehydratase (3.5 mg/ml) in 50 mM Tricine/KOH, pH 8.0. EPR conditions: temperature, 12K; microwave frequency, 9419 MHz; microwave power, 2.6 mW; modulation amplitude, 0.638 mT.

relaxation broadening. These spectral characteristics suggest the presence of a $[3Fe-4S]^+$ cluster in the anaerobic enzyme preparations. Particularly the axial signal around $g = 2.01$ of the enzyme in Tricine (Fig. 1, trace B) is similar to the $[3Fe-4S]^+$ signals of mitochondrial aconitase and many ferredoxines [5,6].

Anaerobic preparations of L-serine dehydratase exclusively exhibited $[3Fe-4S]^+$ signals, the intensities of which corresponded to only 1% of the total enzyme concentration. Upon incubation of L-serine dehydratase under air the line shapes of the signals observed in the different buffers did not change. The signal intensities, however, increased successively and correlated with the decrease in enzymatic activity (Table 1). In addition, a new signal at $g = 4.3$ appeared, characteristic of high-spin Fe^{3+}. Oxidation of L-serine dehydratase with a 2-fold
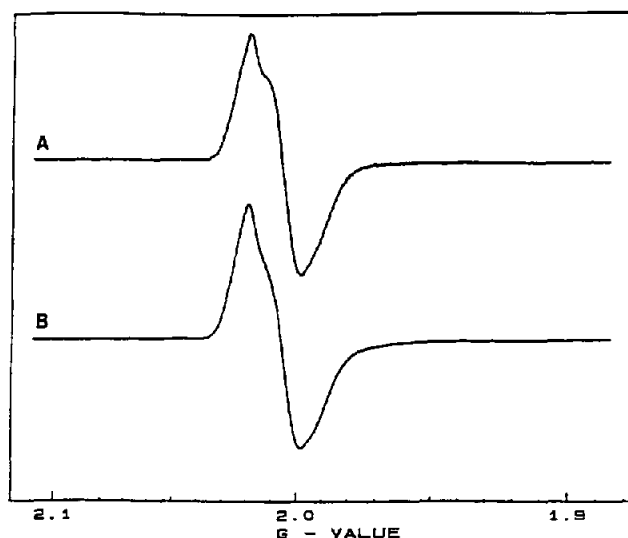

Fig. 2. EPR spectra of L-serine dehydratase from *P. asaccharolyticus* in different buffers after incubation with L-serine. (A) L-Serine dehydratase (0.8 mg/ml) in 50 mM potassium phosphate, pH 7.5, and (B) L-serine dehydratase (3.5 mg/ml) in 50 mM Tricine/KOH, pH 8.0, each after incubation with 50 mM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

molar excess of $K_3[Fe(CN)_6]$ yielded spin concentrations accounting for 90% to 100% of the enzyme concentration. At the same time a complete loss of enzyme activity was observed. Taken together, these results suggest that the paramagnetic $[3Fe-4S]^+$ cluster of the inactive enzyme originated from a diamagnetic $[4Fe-4S]^{2+}$ cluster by oxidation and loss of one iron ion as demonstrated for aconitase [7]. Generally, there also exists the possibility of a direct formation of the $[3Fe-4S]^+$ cluster from a $[3Fe-4S]^0$ cluster by oxidation. Such $[3Fe-4S]^0$ clusters are not readily detected by EPR spectroscopy since they have a broad signal at low field, typical of integer spin systems [8]. The fact, that air inactivated L-serine dehydratase could not be reactivated by different reducing agents but only specifically by Fe^{2+} under anaerobic conditions [2], however, indicates the occurrence of a diamagnetic $[4Fe-4S]^{2+}$ cluster in the active enzyme. Direct evidence for the existence of such a cluster could have been obtained by its reduction to the paramagnetic $[4Fe-4S]^+$ cluster. However, neither incubation of L-serine dehydratase with 5 mM sodium dithionite nor photoreduction resulted in the appearance of a typical $[4Fe-4S]^+$ signal or any other EPR signal. In addition, no decrease in enzymatic activity was observed as reported for the reduced $[4Fe-4S]^+$ form of aconitase [7]. We interpret these results to indicate that the reduction of the $[4Fe-4S]^{2+}$ cluster was either too slow or that the redox potential provided by the reduction system was not sufficiently negative.

As previously mentioned, the EPR signals of the $[3Fe-4S]^+$ cluster exhibited varying line shapes dependent on the buffer (Fig. 1). This was also true for different buffers at the same pH. Hence, the buffer ions themselves rather than the pH affect the environment of the $[3Fe-4S]^+$ cluster. In the presence of 50 mM L-serine EPR signals with only one specific line shape were observed, independent of the buffer used (Fig. 2). These observations imply that the $[3Fe-4S]^+$ cluster is exposed to solutes in the aqueous medium but can be shielded by L-serine. Consequently, the substrate binds to L-serine dehydratase most likely in the vicinity of the $[3Fe-4S]^+$ cluster. The intensities of the $[3Fe-4S]^+$ signal in the presence of L-serine corresponded to spin concentrations which again accounted for only 1% of the concentration of the anaerobic enzyme. Upon incubation of these samples under air the intensities of the EPR signals increased to maximally 2% of the enzyme concentration and no loss of enzymatic activity was detected. This indicates that the $[4Fe-4S]^{2+}$ cluster of the active enzyme is protected by L-serine against oxidation and loss of an iron ion.

On the basis of these results we suggest that the $[4Fe-4S]^{2+}$ cluster exerts an essential function in catalysis by direct interac-

Table 1
Correlation of the $[3Fe-4S]^+$ signal intensities with the activities of L-serine dehydratase

Time (hours)	Spin concentration (% of enzyme concentration)	Loss of activity (%)
0	1	0
0.4	15	8
1.0	18	23
24.0	96	100

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.
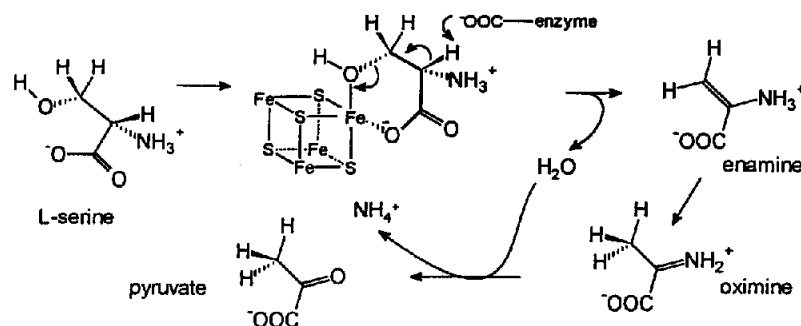
Fig. 3. Proposed mechanism for iron–sulfur cluster-containing L-serine dehydratases. The bold bonds in the complex of L-serine with the iron–sulfur cluster should indicate that they lie above the plane, whereas the hydroxyl oxygen and the front of the cluster lie in the plane.

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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