28

EXAFS of soybean lipoxygenase-1: influence of lipid hydroperoxide activation and lyophilization on the structure of the soybean lipoxygenase non-heme iron active site

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Extended X-ray absorption fine structure (EXAFS) data at the Fe K-edge of the non-heme iron site in native as well as yellow Soybean Lipoxygenase-1 are presented. An edge shift of about 3 eV to higher energy occurs upon oxidation of the native enzyme to the yellow species. Data analysis clearly shows differences in active site structure as a result of this activation of the native enzyme with product peroxide (13(*S*)-HPOD). Curve-fitting on the new data of the native enzyme leads to a coordination sphere that is in good agreement with the earlier proposed active site structure (6±1 N/O-ligands at 2.05-2.09 Å with maximum variance of 0.09 Å), while for the yellow enzyme a shortening in ligand distances and a major change in ligand symmetry seems to occur. Lyophilization of the two species does not seem to affect the iron site structure and gives a much better signal/noise ratio.

Introduction

Lipoxygenases (E.C. 1.13.11.12) are dioxygenases that catalyse the regio- and stereoselective dioxygenation of polyunsaturated fatty acids containing one or more 1*Z*,4*Z*-pentadiene systems. The products are optically active cis, trans-conjugated fatty acid hydroperoxides. In mammals, lipoxygenases are involved in the biosynthesis of physiologically active compounds, the leukotrienes and lipoxins, from arachidonic acid [1]. From the plant enzymes, the soybean type-1 has been thoroughly investigated.

Soybean lipoxygenase-1 (M_r 94.038) contains one gatom of iron per mole of enzyme [2]. It catalyzes the production of 13*S*-hydroperoxy-11*E*-9*Z*-octadecadienoic acid (13(*S*)-HPOD) from linoleic acid. The iron in the native enzyme is high spin FeII (S = 2), while in the yellow enzyme the iron is high spin FeIII (S = 5/2). EPR and other spectroscopic studies have revealed that the iron in yellow lipoxygenase-1 is in an environment with a ligand field of nearly axial symmetry, but not in a porphyrin cofactor [3]. EXAFS studies on the native enzyme show 6±1 nitrogen and/or oxygen-atoms at 2.05-2.09 Å from the iron, with

a maximum variance of 0.09 Å [4]. This has led to a model for the lipoxygenase active site consisting of 4 imidazole-nitrogens at longer distances and 2 oxygens (possibly carboxylate) at a slightly shorter distance from the central iron-atom [4]. EXAFS studies have now been extended to the yellow soybean lipoxygenase-1 and a comparison with native enzyme is presented.

Experimental Section

Lipoxygenase-1 was isolated from soybeans (Williams, American Quality B) according to a previously published procedure [5]. Concentration to approximately 2.5 mM was done with a collodion-bag SM 13200 (Sartorius Membranfilter, Göttingen, FRG). Yellow lipoxygenase-1 was prepared through titration of the native enzyme with 13(*S*)-HPOD until no further increase in absorbance at 330 nm was detectable. Lyophilized enzyme samples were prepared by freeze-drying their 0.1M sodium borate buffer solutions (pH 9.0) on an Edwards model EF03 freeze-dryer for approx. 20 h.

X-ray fluorescence excitation spectra at the Fe K-edge (approx. 7100 eV) were recorded at the EXAFS station of the European Molecular Biology Laboratory (EMBL) Outstation at Hasylab at DESY in Hamburg [6], with an Au-coated mirror, an order-sorting monochromator at 50% harmonic rejection [7], an energy calibration device [8] and a He-cooled cryostat equilibrated at 20-22 K. Measurements on the concentrated as well as the lyophilized enzyme samples were done during main-user time at which the beam was working at an energy of about 7 GeV and a current of 50-100 mA.

Dataprocessing was done by using computer programmes made available by the EMBL [9], while the final analysis of the processed data employed the Daresbury Laboratory EXAFS analysis package including the programme MUFPOT for the ab initio calculation of phaseshifts and backscattering factors, and the fast curved-wave EXAFS simulation and fitting programme EXCURV88 [10,11].

Results and Discussion

The EXAFS of both the native and the yellow substrate-treated enzyme are shown in Fig. 1 (left panel). Apart from the shift in edge energy upon oxidation of the native enzyme to the yellow species some other differences between the two are obvious as well. The "camelback" between 3 $Å^{-1}$ and 5 $Å^{-1}$ which is typical of imidazole backscattering [12] is less pronounced in the fine structure of the yellow enzyme. Nevertheless, the overall beating pattern and amplitude of the fine structure are alike. Comparison of the pre-edge features (Fig. 1 (right panel)) points to the same coordination number for both the native and the yellow enzyme species [13].

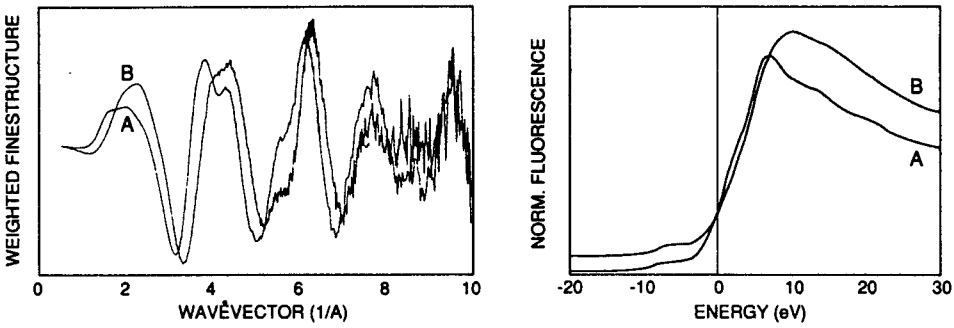

Fig. 1. Left panel: Weighted fine structure (k^3) of lyophilized native (A) and yellow lipoxygenase-1 (B); Right panel: Normalized edge region of lyophilized native (A) and yellow lipoxygenase-1 (B)

Preliminary data analysis and curvefitting on the yellow enzyme data leads to a coordination sphere in which the FeIII is ligated by three imidazole nitrogens and three oxygens, while for the native enzyme the FeII was proposed to be ligated by four imidazole nitrogens and two oxygens [4]. Thus, it seems that

one imidazole is lost as an iron ligand during activation of the enzyme by product peroxide and one oxygen, of unknown origin, is gained. Furthermore, the ligand distances found for the yellow enzyme species are slightly shorter than those found for the native enzyme species.

As can be seen from Fig. 2, lyophilization of both the native and the yellow enzyme species does not seem to affect the iron coordination. Apart from an increase in signal/noise ratio for the lyophilized samples in relation to the frozen solutions, the EXAFS is largely unchanged. The shape and intensity of the pre-edge features (not shown) do not change significantly, suggesting that no alteration of the coordination numbers occurs as a result of the lyophilization procedure.

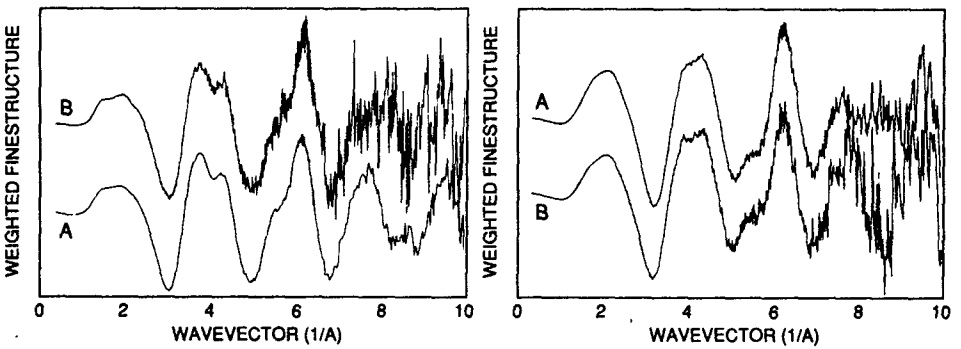

Fig. 2. Left panel: Weighted fine structure (k^3) of native enzyme: lyophilized (A) and frozen solution (B); Right panel: Weighted fine structure (k^3) of yellow enzyme: lyophilized (A) and frozen solution (B)

This result implies that if there is indeed exchangeable H_2O coordinated to the iron as was reported [14], it is not lost upon lyophilization.

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