Three-dimensional structure of human 5-LOX in solution: new insights from SAXS analysis

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Lipoxygenases (LOXs) form a heterogeneous family of fatty acid dioxygenases that are widely distributed in plants and animals [1, 2]. Human 5-LOX is implied in the biosynthesis from arachidonic acid (AA) of leukotrienes, lipid mediators of inflammation [2].
We showed that plant and mammalian lipoxygenases have different degrees of conformational flexibility and thermal stability [3]. 5-LOX activity is short-lived, apparently in part because of an intrinsic instability of the enzyme. The crystal structure of a mutant human 5-LOX, where substitutions have been introduced to stabilize the enzyme, has been solved at 2.4 Å resolution [4].
In order to determine the oligomerization state of the native protein in solution and possible differences with the crystal structure of the 5-LOX mutant, we performed a combined Small Angle X-Ray Scattering (SAXS) and Size Exclusion-HPLC (SE-HPLC) study on wild-type human 5-LOX. Unlike soybean LOX-1, that we previously showed by SAXS to be organised as a stable monomer [5], the SE/HPLC profile of human 5-LOX suggests the presence of three different protein species. The analysis of the SAXS scattering pattern of each chromatographic peak revealed that 5-LOX in solution is present mostly as a homodimer. In particular, we revealed the presence of two dimeric species differing for their hydrodynamic volume, as well as of monomers (at a low percentage). No higher aggregation states were detected. Here we present the 3D model of human 5-LOX in solution.
It has been recently suggested that 5-LOX could form a dimeric complex, where one monomer catalyzes the generation of 5-HPETE, which is then transferred to the other monomer for the formation of leukotrienes [6]. Our results confirm these observations and open an interesting question on the possible effectors that might modulate monomer/monomer interactions, and hence monomer/dimer equilibrium, leading to a specific functional regulation of 5-LOX *in vivo*.

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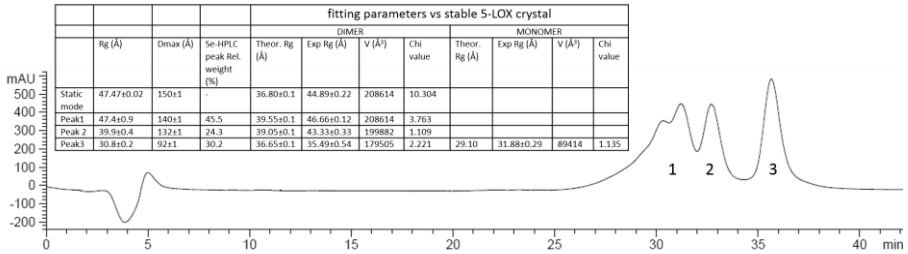

				fitting parameters vs stable 5-LOX crystal							
				DIMER				MONOMER			
	Rg (Å)	Dmax (Å)	Se-HPLC peak Rel. weight (%)	Theor. Rg (Å)	Exp Rg (Å)	V (Å³)	Chi value	Theor. Rg (Å)	Exp Rg (Å)	V (Å³)	Chi value
Static mode	47.47±0.02	150±1		36.80±0.1	44.89±0.22	208614	10.304				
Peak1	47.4±0.9	140±1	45.5	39.55±0.1	46.66±0.12	208614	3.763				
Peak 2	39.9±0.4	132±1	24.3	39.05±0.1	43.33±0.33	199882	1.109				
Peak3	30.8±0.2	92±1	30.2	36.65±0.1	35.49±0.54	179505	2.221	29.10	31.88±0.29	89414	1.135

Figure 1. SE-HPLC elution profile of wild-type human 5-LOX. Inset: Overall structural SAXS parameters of each peak and of the low-resolution 3D models.

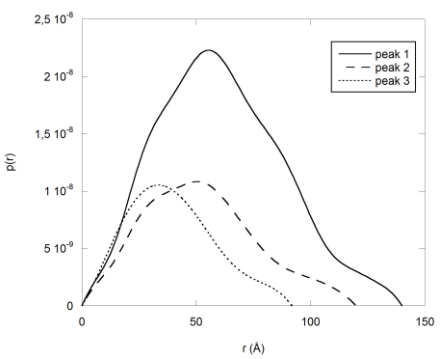

Figure 3. p(r) functions of the three peaks of human 5-LOX separated by SE-HPLC.

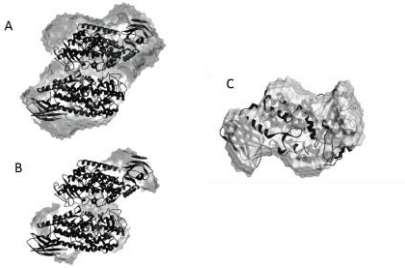

Figure 4. Low-resolution 3D models of the three 5-LOX species detected in solution. The models were calculated from the SAXS profiles with DAMAVER (ATSAS suite [7]). A: Peak 1 (dimer); B: peak 2 (dimer); C: peak 3 (monomer) superposed to the stable 5-LOX crystal structure represented as black ribbons.