

SEC-complex-down approaches with functional O₂-affinity assay: Correlation between the higher order structure of bird hemoglobin homologues and their function.

Wednesday, August 27, 2025 11:00 AM (5 minutes)

Hemoglobin is a tetrameric protein responsible for the blood oxygen transport. Any abnormalities in hemoglobin structure can lead to serious health outcomes. Although very well characterized in humans, it has been scarcely studied in the case of birds. Some studies have been carried out at the globin level without providing further evidences about the native structure of the complex. In this context, we propose for the first time the combination of size-exclusion chromatography with complex-down mass spectrometry for the straight correlation of binding stoichiometry of subunits, their primary structure, and the identity of different cofactors. Complex-up analysis were carried out to decipher the identity of the individual constituents of each tetramer population by providing increased energy in the mass spectrometer ion source. Thus, it was concluded that mass differences between the three tetramers were due to the substitution of alpha subunits. In addition, one cofactor was also identified for the first time linked to the tetramers.

Information about subunit sequence characterization and cofactor identification were afforded through SEC-complex-down approach (pMS3) to isolate and fragment the different constituents. Fragmentation of subunits was achieved by combining various fragmentation methods leading to an overall sequence coverage of 98, 94, and 96% for aA, aD, and b subunits, respectively. The pMS3 approach allowed to determine the presence of IP5 cofactor, which is known to regulate O₂-hemoglobin affinity in birds.

Finally, the three tetramers were collected separately upon SEC separation to record O₂-affinity data for each tetramer. The functional data clearly showed affinity differences between the three populations, clearly pinpointing for the first time that bird hemoglobin affinity correlated with the number of aD subunits in the tetrameric structure. Altogether, the results from the SEC complex-down analysis in combination with O₂-affinity data could find application in evolution analysis, environmental adaptation, or different clinical contexts.

User consent

yes

Author: LETISSIER, Léa (LSMBO - CNRS (Université de Strasbourg))

Co-authors: Mrs SCHAEFFER, Christine (LSMBO - CNRS (Université de Strasbourg)); Mr BERTILE, Fabrice (DEPE - CNRS (Université de Strasbourg)); Mr CRISCUOLO, François (DEPE - CNRS (Université de Strasbourg)); HERNANDEZ ALBA, Oscar (CNRS); Mrs CIANFÉRANI, Sarah (LSMBO - CNRS (Université de Strasbourg)); Mrs NABIYEVA, Turkan (LSMBO - CNRS (Université de Strasbourg))

Presenter: LETISSIER, Léa (LSMBO - CNRS (Université de Strasbourg))

Session Classification: Lightning Talks