The Role of Hemoglobin’s C-terminal Region: the Work of Eraldo Antonini

Studies on Carboxypeptidase Digests of Human Hemoglobin

The Effect of Oxygenation on the Rate of Digestion of Human Hemoglobins by Carboxypeptidases

Eraldo Antonini (1931–1983) received his degree in Medicine and Surgery from the University of Rome in 1955. He then began studying hemoglobin and myoglobin with Alessandro Rossi-Fanelli at the Institute of Biochemistry of the University of Rome and at the Regina Elena Institute for Cancer Research.

In 1961, Antonini and Rossi-Fanelli published a paper describing the effect on human hemoglobin’s activity when the C-terminal amino acid residues are removed from the molecule’s α and/or β chains. This paper is reprinted here as a Journal of Biological Chemistry (JBC) Classic. In the study, the scientists used carboxypeptidase A to digest the C-terminal tyrosine and histidine on the molecule’s β chain and carboxypeptidase B to remove the C-terminal lysine, tyrosine, and arginine on the molecule’s α chain. The resulting protein appeared intact but had an increased oxygen affinity, lowered cooperativity, and dramatically reduced Bohr effect.

This observation inspired Max Perutz, who wrote: “Several years later, my electron density maps showed that these residues form salt bridges with neighboring subunits in deoxyhemoglobin which get broken on transition to oxyhaemoglobin. Remembering Antonini’s observation, I realized at once that these bridges must represent the additional bonds between the subunits in the T structure predicted by Monod, Wyman and Changeux’s theory of allostery. Antonini had also demonstrated that the release of Bohr protons is colinear with oxygen uptake. When Kilmartin’s and my work proved that most of the Bohr protons originate from the salt bridges, it became clear to me that oxygen uptake is linked to the rupture of these bridges” (1).

In the second JBC Classic reprinted here, Antonini follows up on the first paper by doing a reciprocal experiment in which he looks at differences in digestion rates of oxy- and deoxyhemoglobin, reasoning, “If enzymatic modification can affect conformation and changes of conformation resulting from combination with ligand (oxygen), one might expect that the rate of attack on the hemoglobin by the enzymes should depend on the presence or absence of ligand; this would determine conformation, and conformation, in turn, would control the rate.” Again using carboxypeptidases A and B, he showed that the rate of digestion is different for the oxy- and deoxy- forms of the molecule, indicating a differential accessibility of the C-terminal residues to these enzymes.

This work was later extended and perfected by John V. Kilmartin on a suggestion by Perutz, who pointed out the crucial role of the C-terminal residues for the molecular mechanism of cooperativity and the Bohr effect. Kilmartin was able to differentiate the role of the C-terminal histidine from that of tyrosine by preparing and characterizing a modified hemoglobin devoid of histidine.
Over the next several years, Antonini continued to study hemoglobin, looking at the properties of the α and β chains, the acid-base equilibria of hemoglobin, the Bohr effect and its dependence on temperature, the oxidation-reduction equilibria, ligand-induced conformational changes in hemoglobin, and the kinetics of the reaction of myoglobin and hemoglobins with ligands. This work culminated in the publication of *Hemoglobin and Myoglobin in Their Reactions with Ligands* in 1971 (2), which was a landmark in the field.

In the 1970s, Antonini expanded his scientific interests and started focusing on electron-transfer metalloproteins (such as cytochrome c oxidase) and on proteolytic enzymes. He eventually became Professor of Molecular Biology at the University of Camerino and was later made Professor of Chemistry and Director of the Institute of Chemistry in the Faculty of Medicine and Surgery at the University of Rome. He also received the Feltrinelli Prize from the Accademia Nazionale dei Lincei in 1974.

One of Antonini’s coauthors on the two JBC Classics reprinted here is JBC Classic author Jeffries Wyman (3) who came to Rome in 1961 for a week-long visit and ended up remaining for 25 years and working with Antonini. This collaboration produced a series of outstanding papers and conceptual advancements that have had a long lasting influence on protein chemistry.1,2

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REFERENCES


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1 Thank you to Maurizio Brunori for providing background information for this JBC Classic.

2 Biographical information on Eraldo Antonini was taken from Ref. 4.
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