In 1966 as a chemistry student in the Biochemistry Department of Loránd Eötvös University, Budapest, I joined András Mühlrad's group. They had found that diethylpyrocarbonate (DEP) reacts not only with amino groups but also with other protein side-chains at slightly alkaline pH. These reactions led to the formation of the corresponding carbethoxy derivatives, resulting in an increase of absorbance in the ultraviolet range.

In 1967 I had the opportunity to do my master's degree work at the Institute of Biochemistry (now the Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences) in Pál Elődi's group. The subject that we planned to elaborate, supervised by Susan Libor, was how to make the carbethoxylation specific for histidyl residues of proteins. Heretofore, the only method for histidyl modification was photooxidation. However, it was known that it caused the irreversible denaturation of proteins.

At that time, I believed I was working on a very important scientific problem, simply because everything that happened in the lab seemed important to me. After eight months of work, just after getting my degree, we published the results in three pages in a national scientific journal. It was a mistake, as I reflect now, not to publish in a well-known international journal. This might account for the fact that our paper has been cited rarely in the last 5 to 10 years as the original reference on this topic.

Subsequently, we extended the method for isolation of peptide-containing carbethoxy-histidyl residue and applied it to the investigation of the catalytic role of histidyl residues in glycolytic enzymes. The usage of DEP in the study of structure-function relationships in enzymes has become more widespread since the synthesis of its isotope derivatives. Nowadays, it is used in many different fields, such as nucleic acid research and plant physiology.

I believe that my first publication after graduate school became a Citation Classic due to the nature of the work and its analytical utility. The method we introduced for identification of histidyl residues in the active site of enzymes is both simple and effective. And, I think, independently of which paper is cited as the original, this method is beneficial to the work of many protein chemists and enzymologists, and may continue to be so until side-directed mutagenesis techniques become applicable in this area.

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