

**RATE CONSTANTS OF THE REACTION OF HYDROXYL RADICALS  
( $^{\circ}\text{OH}$ ) WITH ALCOHOL DEHYDROGENASE (ADH) AND  
GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH)**

ALEKSANDRA KOWALCZYK, ANETA DUDEK

and MIECZYSLAW PUCHALA

Department of Molecular Biophysics, University of Łódź, Poland

Rate constants of reactions of the products of water radiolysis with proteins are the basic parameters in studying free-radical reactions. The most highly reactive radical, of a very high redox potential is the hydroxyl radical. It demonstrates very strong oxidation characteristics, due to which it reacts very rapidly with most compounds occurring in cells and tissues. The result of such reactions can be damage to nucleic acids, structural and functional changes in proteins, as well as peroxidation of lipids. Rate constants of the reactions of  $^{\circ}\text{OH}$  radicals with proteins have a value of the order of  $10^9 - 10^{11} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ .

Rate constants of reactions of  $^{\circ}\text{OH}$  radicals were estimated for two enzymes: alcohol dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase. These rate constants were determined by the steady-state competitive reaction method, measuring the inactivation of enzymes irradiated in the presence and absence of ethanol, which competed with the enzyme for  $^{\circ}\text{OH}$  radicals. The concentration of alcohol in irradiated solutions ranged from  $10^{-4} \text{ mol/dm}^3$  to  $10^{-2} \text{ mol/dm}^3$ . Protein preparations at a concentration of 0.1 mg/ml were irradiated with X rays in phosphate buffer, pH 7.0.

The rate constants of  $^{\circ}\text{OH}$  radical reactions were calculated on the basis of the radiation yield of inactivation estimated from the dose-effect curves assuming the rate constant of  $^{\circ}\text{OH}$  radical reaction with ethanol to be  $1.9 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ .