^{1}H-NMR studies on the mechanism of action of the glyoxalase system

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Methylglyoxal is known to inhibit cell division and to block DNA and protein synthesis. Its oxidation by the glyoxalase (GLO) enzyme system enables the cells to proliferate again. The GLO system consists of two enzymes, GLO I (EC 4.4.1.5) and GLO II (EC 3.1.2.6), with reduced glutathione (GSH) as a cofactor. The real substrate of this enzyme system is not known yet, however, it acts very well on methylglyoxal. In this way, methylglyoxal, linked with GSH to a hemimercaptal, is transformed to a thioester by GLO I and oxygen. This complex is separated, then, by GLO II to GSH and d-lactic acid.

Using ^{1}H-nuclear magnetic resonance (NMR) spectroscopy, the broadening of the glutathione signals indicates the hemimercaptal complex between glutathione and methylglyoxal. In this complex, which is formed prior to an enzymatic action of the glyoxalase system, the lactoyl H-1 resonance is observed at about 3.50 ppm and the ß-CH_2 of the cysteinyl group exhibits a doublet at about 3.41 ppm. After application of GLO I the H-1 resonance disappears. The ß-CH_2 signal is shifted downfield to 3.78 ppm due to the oxidation on the lactoyl C-1 and is splitted into eight lines indicating a restricted rotation ability in this part of the molecule. Lactic acid produced by the subsequent application of GLO II is identified by its CH_3-doublet at 1.82 ppm and its CH-quartet near 4.77 ppm. Simultaneously with the lactate resonances those of reduced glutathione appear again. Hence, it is shown that the glyoxalase action can be followed by means of the NMR technique and molecular interpretations of this mechanism can be given.