Application of Dansylglycine for Determination of Myeloperoxidase Halogenating Activity

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INTRODUCTION: The enzyme myeloperoxidase (MPO), through its halogenating activity, is able to catalyze the oxidation of chloride (Cl^-) and bromide (Br^-). The oxidizing agents formed, hypochlorous acid (HOCl) and hypobromous acid (HOBr) are highly reactive and involved in chronic inflammatory diseases. The determination of halogenating activity of MPO is not trivial, because it is able to catalyze the oxidation of various organic compounds by peroxidase mechanism. **OBJECTIVE:** In these work we aimed to apply the fluorescent probe dansylglycine (DG) to determine the specific halogenating activity of MPO. **MATERIAL AND METHODS:** The reaction medium, composed of DG, Cl^-, Br^-, H_2O_2 and MPO, was studied by fluorimetry (λ_{ex} = 340 nm and λ_{em}= 575 nm). The consumption of H_2O_2 was monitored using an amperometric detector and a hydrogen peroxide-specific electrode. **RESULTS AND DISCUSSION:** The consumption of DG was dependent of the presence of Cl^- and Br^-. These results confirmed the specificity of the method for the halogenating activity of MPO. The enzyme kinetic studies showed a linear correlation between the enzyme concentration and the rate of DG consumption. The methodology was also applied to determine the effect of specific inhibitors of MPO. By using the electrochemical detector to monitor the consumption of H_2O_2 it was possible to demonstrate that the addition of Br^- significantly increases the reaction rate. It was also observed that in the absence of substrate for MPO, it is able to degrade H_2O_2 through its catalase mechanism. **CONCLUSION:** In conclusion, this methodology is sensitive and specific for halogenating activity of MPO and could be applied for determination of inhibitors of the enzyme.

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