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DETECTION OF THE BROMINATING ACTIVITY OF MYELOPEROXIDASE USING FLUORESCEIN

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A study was carried out on the spectralluminescent properties of fluorescein after its reaction with various reactive oxygen and halogen species ($O_2^{\bullet-}$, H_2O_2, HOCl, HOBr, HOSCN, N-chloramine, taurine N-chloramine, and taurine N-bromamine) as well as in the myeloperoxidase (MPO)–H_2O_2–Cl^-/Br^-/SCN^- system. Reaction with only HOBr or with the MPO–H_2O_2–Br system turns fluorescein into a compound with an absorption maximum at 518 nm. The fluorescence maximum is recorded at 540 nm when excited at 520 nm, corresponding to eosin Y (brominated fluorescein). Conditions with phosphatebuffered saline (PBS) at pH 7.4 containing 137 mM NaCl, 5 mM fluorescein, 15–30 mM NaBr, and 25–50 mM H_2O_2 were found to be optimal for detecting HOBr in solution. A qualitative method for determining the brominating activity of MPO in vitro has been proposed. This method was used to study the effect of physiological and synthetic inhibitors as well as reactive oxygen and halogen species scavengers on the brominating activity of MPO. Our results indicate that fluorescein holds promise for use in a fluorescent method for detecting the brominating activity of mammalian hemecontaining peroxidases.

Keywords: *myeloperoxidase, fluorescein, eosin Y, hypobromous acid, brominating activity.*

Introduction. The family of mammalian hemecontaining peroxidases (donor:H_2O_2–oxidoreductase, KF 1.11.1.7) consists of four major enzymes: myeloperoxidase (MPO), eosinophil peroxidase, lactoperoxidase, and thyroid peroxidase [1, 2]. Bathish [2] and Dunford [3] have recently discovered another two representatives of this family: peroxidazine and peroxidazinelike protein. Peroxidases possess peroxidase activity as well as the capacity to catalyze the oxidation of halides (Cl^-, Br^-, and I^-) and pseudohalides such as SCN^- to give hypohalous acids (HOX, where X is a halogen or pseudohalogen) [4]. HOCl and HOBr, the most common hypohalous acids, are strong oxidizing agents capable of reacting with many biologicallyimportant compounds such as nucleic acids, carbohydrates, amino acids, proteins, and lipids [5, 6]. Due to this property, peroxidases, on one hand, perform a bactericidal function, protecting the organism from pathogens and, on the other hand, participate in a whole series of processes damaging the cells and tissues of host organisms, leading to oxidative/halogenative stress [7–10]. These findings have led to an active search for methods to detect the formation of hypohalous acids, primarily HOCl as the most common such species encountered *in vivo.*

Although HOBr is similar in its physicochemical properties to HOCl, it has attracted relatively little attention. The formation of HOBr in living organisms is considered less likely than HOCl since the concentration of bromide, from which HOBr is formed, is about a thousand times lower in human blood than for chloride. Nevertheless, the reactivity and role in pathological processes of HOBr differ from those of HOCl. For example, peroxidazine catalyzes the brominedependent formation of crosslinks in the synthesis of collagen IV and connective tissues, carrying out an important physiological function [2]. In the presence of physiological concentration of Br^- ion (~100 μM), there is a sharp increase in the yield of brominated human serum albumin (HSA) formed in reactions catalyzed by MPO [11]. Suzuki [12] and Panasenko [13] have detected

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