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A fluorescence reaction for the sensitive quantification of orotic acid

◯尹　晟[1], 柴田　孝之[1], 藤田　順也[1], Shpend DRAGUSHA[1], 椛島　力[1], 甲斐　雅亮[1,2]
([1]長崎大院医歯薬, [2]長崎大グローバルCOEプログラム)

[Introduction] Orotic acid is an intermediate in the biosynthetic pathway of pyrimidine nucleotides. Markedly increased level of orotic acid in urine is found in some disorders of metabolism such as urea cycle, which is called orotic aciduria. Determination of orotic acid in urine has been applied for diagnosing the errors in urea cycle. Recently, we developed a specific fluorescence (FL) reaction for pyrimidine nucleobases using benzamidoxime (BAO) as a fluorogenic reagent. The reaction requires heating at 100ºC for 5 min, and the FL intensity is simply measured with a spectrofluorometer. Although the reaction produces FL with uracil, cytosine and orotic acid, we found that the selectivity of the present reaction varies dramatically by changing the reaction conditions. In this study, we have attempted to develop a specific fluorogenic reaction for orotic acid, and to apply the reaction for quantification of orotic acid in urine samples.

[Methods] A mixture of nucleobases, BAO, $K_3[Fe(CN)_6]$ was heated at 25-100°C for 0.5-60 min in the presence of various bases, and the resulting FL intensity was measured with a spectrofluorometer at the excitation and emission wavelengths of 330 nm and 410 nm, respectively.

[Results and Discussion] We found that the FL reaction was preferable for orotic acid when the basicity of the reaction mixture is low. The highest FL intensity was obtained when the mixture of 1.25 mM BAO, 2.0 mM $K_3[Fe(CN)_6]$ and 20 mM K_2CO_3 was heated at 100°C for 5 min. Orotic acid produced approximately 6 times stronger FL intensity than that of uracil or cytosine under these conditions, and the other nucleobases such as thymine, guanine and adenine produced no FL. This method would be useful for diagnosing orotic aciduria through detection of orotic acid in urine.