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Syringaldazine

New Attractive Electron Donor of Prostaglandin H Synthase

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ABSTRACT

The prostaglandin H synthase (PGHS) reaction has been studied with syringaldazine as the electron donor. Kinetic parameters of the reaction agreed with those for commonly used electron donors. The sensitivity of the PGHS activity detection in the presence of syringaldazine increases 10 times. A sensitive spectrophotometric assay for polyunsaturated fatty acid (PUFA) detection using syringaldazine as a new electron donor has been proposed.

Index Entries: Prostaglandin H synthase; syringaldazine; spectrophotometric assay; polyunsaturated fatty acids.

INTRODUCTION

Prostaglandin H synthase (PGHS) (EC 1.14.99.1) is the first and rate-limiting enzyme in prostaglandin synthesis (*1,2*). The enzyme catalyzes the conversion of a polyunsaturated fatty acid (PUFA) into prostaglandin H. The enzyme activity is dependent on hemin, which acts as a prosthetic group (*3*), and an electron donor (*4*), which is required for prostaglandin G conversion into prostaglandin H.

Various physicochemical methods are used for PGHS activity detection. Among them are polarographic assay (*5*), fluorometric assay (*6*), and spectrophotometric assay (*7*). It has been found (*6*) that the sensitivity of the fluorometric assay using homovanillic acid as the electron

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donor is, by one order of magnitude, higher than that of spectrophotometric or polarographic techniques. However, the fluorometric assay is not a routine procedure and requires carefully prepared reagents. The spectrophotometric technique is easy and accessible. To increase spectrophotometric assay sensitivity for the PGHS activity, we suggest syringaldazine as a new electron donor.

The aim of the present work was to design a simple and effective method of total arachidonic and eicosapentaenoic acid detection in human blood based on the PGHS reaction. Detection of PUFAs in human blood, in the other biosources and microbiological synthesis systems, is important because of their being precursors of physiologically active compounds (prostanoids, leukotrienes, and lipoxins) (*4*,*8*). It has been found (*9*) that PUFA concentrations in blood of patients with cardiovascular diseases are abnormal and correlate with disease severity.

MATERIALS AND METHODS

Materials

Arachidonic and eicosapentaenoic acids, hemin, and syringaldazine were from Sigma (St. Louis, MO); Tris was from Serva (Heidelberg, Germany); Tween 20 was from Ferak (Berlin, Germany).

Solubilized microsomes from sheep vesicular glands were the source of PGHS obtained as described previously (*3*).

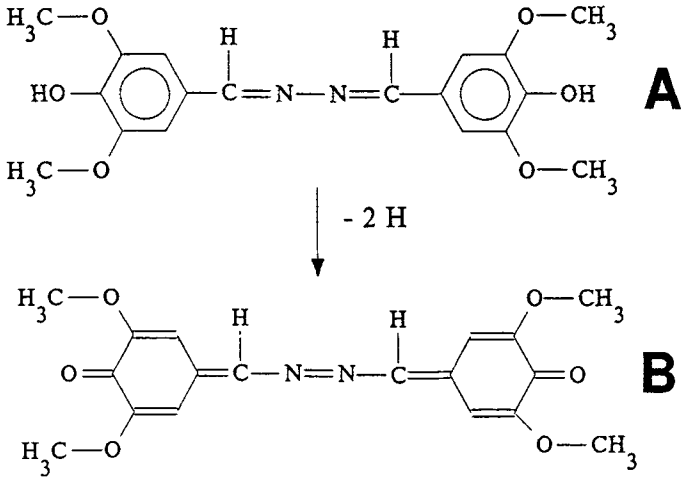
Methods

PGHS activity was measured by a spectrophotometric assay using syringaldazine as a substrate and a Hitachi-557 (Japan) spectrophotometer to register the absorption spectra.

Arachidonic or eicosapentaenoic acid was added to the reaction mixture containing 2 μM hemin, 60 μM syringaldazine, 0.05 mg/mL PGHS, and 0.1% Tween 20 in 50 mM Tris-HCl buffer (pH 7.5).

RESULTS AND DISCUSSION

We have successfully employed syringaldazine, laccase, and peroxidase substrate (*10*) as the electron donor in the PGHS reaction. The oxidation of syringaldazine (**A**) results in a product (**B**) with ϵ_{530} = 65,000 M^{-1}/cm (*11*):



PGHS has been shown to have a broad substrate specificity with respect to the electron donor (*12,13*). Measurement of the concentrations of oxidized electron donors underlies the spectrophotometric procedure for determination of this enzyme's activity. However, the capability of this method is limited by the low molar absorption coefficients of the compounds that form.

It seemed tempting to us to use syringaldazine as the electron donor in the PGHS reaction for the determination of enzyme activity, since the oxidized form of syringaldazine displays strong absorption. It is about 10–15 times higher then that for oxidized forms of commonly used electron donors, such as adrenaline, potassium ferrocynide, and hydroquinone (*4*). The violet color appears immediately. Therefore, the reaction can be used as an express method.

Preliminarily, we have determined the optimum concentration of syringaldazine for the PGHS reaction proceeding. It was about 60 μM (Fig. 1).

We have investigated kinetics of the reaction of PGHS with arachidonic acid (Fig. 2), and eicosapentaenoic acid (Fig. 3) in the presence of syringaldazine. The kinetic parameters of the PGHS reaction with arachidonic acid have been calculated:

$$K_M = (2.27 \pm 0.33) \times 10^{-5} M \quad (1)$$

$$V_{max} = (1.4 \pm 0.05) \times 10^{-5} M/\text{min} \quad (2)$$

The kinetic parameters of the reaction agreed with those for commonly used substrates (*4*). The Michaelis constant for eicosapentaenoic acid was $(2.13 \pm 0.83) \times 10^{-3} M$.

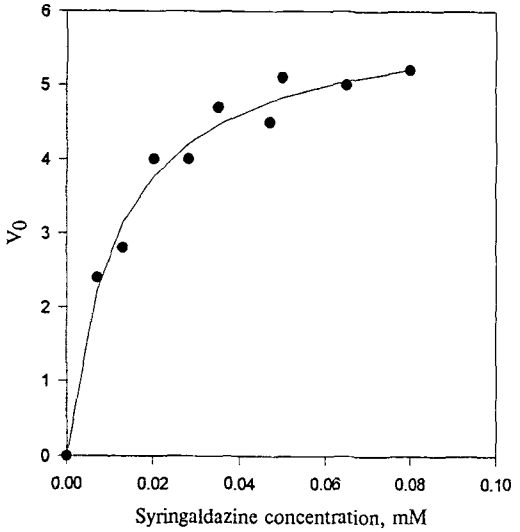


Fig. 1. The rate of the PGHS reaction vs syringaldazine concentration. Conditions: 50 m*M* Tris-HCl, pH 7.5, 0.1% Tween 20, 0.05 mg/mL PGHS, 2 μM hemin, and 0.1 m*M* AA.

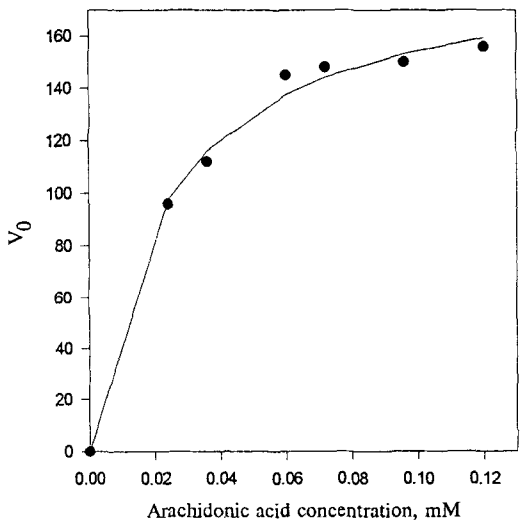


Fig. 2. The rate of the PGHS reaction vs arachidonic acid concentration. Conditions: 50 m*M* Tris-HCl, pH 7.5, 0.1% Tween 20, 0.05 mg/mL PGHS, 2 μM hemin, and 60 μM syringaldazine.

The sensitivity of the spectrophotometric detection of the PGHS reaction with syringaldazine as the electron donor increases at least 10 times, which makes it possible to determine low concentrations of PUFAs. We suppose that the method can be applied to design an express test for PUFA detection in medicine and microbiology.

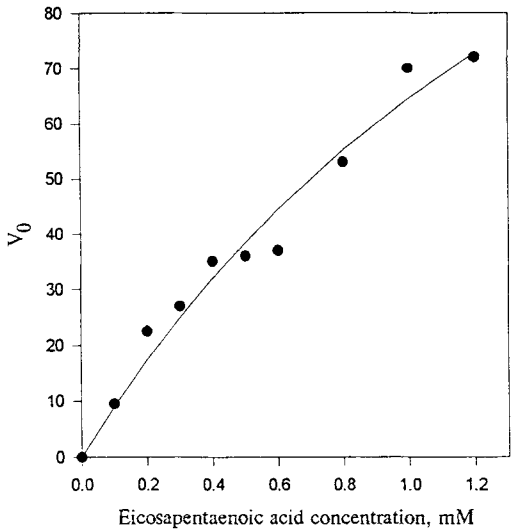


Fig. 3. The rate of the PGHS reaction vs eicosapentaenoic acid concentration. Conditions: 50 m*M* Tris-HCl, pH 7.5, 0.1% Tween 20, 0.05 mg/mL PGHS, 2 μM hemin, and 60 μM syringaldazine.

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REFERENCES

1. Hamberg, M., Svensson, J., and Samuelsson, B. (1973), *Proc. Natl. Acad. Sci. USA* **70,** 899-903.
2. Nugteren, D. H. and Hazelhof, E. (1973), *Biochim. Biophys. Acta* **326,** 448-461.
3. Van der Ouderaa, F. J., Buytenhek, M., Nugteren, D. H., and Van Dorp, D. A. (1977), *Biochim. Biophys. Acta* **487,** 315-331.
4. Varfolomeev, S. D. and Mevkh, A. T. (1985), *Prostaglandins—Molecular Bioregulators.* Moscow University, Moscow.
5. Achmedov, R. M., Mevkh, A. T., and Kulys, J. J. (1984), *Anal. Chim. Acta* **166,** 301-304.
6. Mevkh, A. T., Sud'ina, G. F., Golub, N. B., and Varfolomeev, S. D. (1985), *Anal. Biochem.* **150,** 91-96.
7. Takeguchi, G. and Sih, C. J. (1972), *Prostaglandins* **2,** 169-173.
8. Nelson, N. A., Kelly, R. C., and Johnson, R. A. (1982), *Chem. and Eng. News* **16,** 30-44.
9. Mevkh, A. T., Yuskovich, A. K., Duzhenko, V. S., Li, E. D., and Lakin, K. M. (1990), *Kardiologiya* **9,** 54-57 (in Russian).
10. Grassin, C., and Dubourdieu D. (1989), *J. Sci. Food and Agriculture* **48,** 369-376.
11. Bauer, R. and Rupe, C. (1971), *Anal. Chem.* **43,** 421.
12. Ohki, S., Ogino, N., Yamamoto, S., and Hayaishi, O. (1979), *J. Biol. Chem.* **254,** 829-836.
13. Ogino, N., Ohki, S., Yamamoto, S., and Hayaishi, O. (1978), *J. Biol. Chem.* **253,** 5061-5068.