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Voltammetric and capillary electrophoretic study of scavenger kinetics of methylglyoxal by antidiabetic biguanide drugs

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ABSTRACT

A novel electroanalytical approach is used to get insight into scavenger kinetics and mechanism of methylglyoxal (MG) by biguanides metformin, phenformin and 1-phenylbiguanide under the physiological conditions (pH 7.41 and 37 °C). The approach is based on monitoring the time profiles of concentrations of the protonated biguanides by ion transfer voltammetry (ITV) at the polarizable room-temperature ionic liquid membrane. Effects of the reactant's concentrations and pH lead us to propose a mechanism including an acid-base reaction of biguanide, which separates the parallel reactions of the protonated and deprotonated biguanide with methylglyoxal. Reaction rate increases considerably in the sequence metformin < phenformin < 1-phenylbiguanide. Kinetic data confirm the previous finding that scavenging of methylglyoxal by metformin is a relatively slow process. Optimization of the background electrolyte enables to use capillary electrophoresis (CE) to detect simultaneously the protonated biguanide and the MG anion in the reaction mixture. CE analysis then yields the mole-to-mole ratios of the reacted biguanides to MG in the range 0.6–0.7 pointing to the simultaneous formation of a 1:1 and a 1:2 reaction products. Usefulness of ion voltammetry or amperometry for further kinetic studies of biguanides, as well as for their assays, can be envisaged.

1. Introduction

Methylglyoxal (MG, Fig. 1A) has been recognized as the most powerful glycating agent in the formation of so-called advanced glycation end-products (AGEs), which are related to diabetes and other age-related diseases [1]. Several pharmacological preparations have been examined for inhibiting the formation of AGEs by scavenging the reactive α-dicarbonyl species. In this respect, the substituted biguanides, such as metformin (MF, Fig. 1B) or phenformin (PF, Fig. 1C), have turned out to be very efficient [2]. A more recent study supported previous finding that MF can reduce plasma MG in type 2 diabetic patients [3]. However, the study also indicated that this reduction is not only due to the scavenging of MG by MF, but also due the restoration of activity of enzyme Glyoxalase 1 in the major route of MG detoxification [3]. Kinetics of the reaction between MG and MF in an aqueous solution has been previously investigated under physiological conditions (pH 7.4, 37 °C) by monitoring the time profiles of the concentrations of MF and of several reaction products by HPLC/MS [4], and by absorption spectroscopy [5]. The MS analysis [4] indicated the intermediate formation of a 1:1 linear addition product (LAP, Fig. 1D), which is transformed into a cyclic condensation product, triazepinon (TP, Fig. 1E). A comparable amount of an addition product of TP and the second molecule of MG was also found [4]. An analysis of the early stage adducts of the reaction between MG and MF provided ^{1}H NMR spectrum and MS data indicative of a mixture of hydroimidazolone isomers and tautomers, while TP was anticipated as a slowly formed product [5]. More recently, an imidazolinone (imidazolone) derivative (IMZ, Fig. 1F) was identified as the primary product on the basis of the MS and ^{13}C and ^{1}H NMR analyses, while the formation of TP was questioned [6]. It is noteworthy that all mechanistic considerations have ignored a strong basicity of biguanides leading to their protonation in the aqueous solutions in a broad range of pH < 11 [7,8]. In particular, the following values of the acidity constants pK_a = 13.85 [8], 13.27 [8] and 10.76 [7] were reported for the monoprotonated biguanides MFH^+, PFH^+ and PBH^+ (PB = 1-phenylbiguanide, Fig. 1G), respectively.

In the present study we developed a novel electroanalytical approach, which is based on the ion transfer voltammetry of the protonated biguanides at a polarized ionic liquid (IL) membrane [9]. The approach allowed us to monitor the time profiles of concentrations of the protonated MF, PF or PB under the physiological conditions (pH 7.41, 37 °C), and to evaluate the corresponding rate constant. Capillary electrophoretic detection of both the protonated biguanides and the ionized MG was used to determine the reaction stoichiometry. Apart from presenting an analysis of kinetics and mechanism of the reaction, we shall demonstrate that the reaction rate depends on the biguanide structure and temperature. Observed effect of the solution

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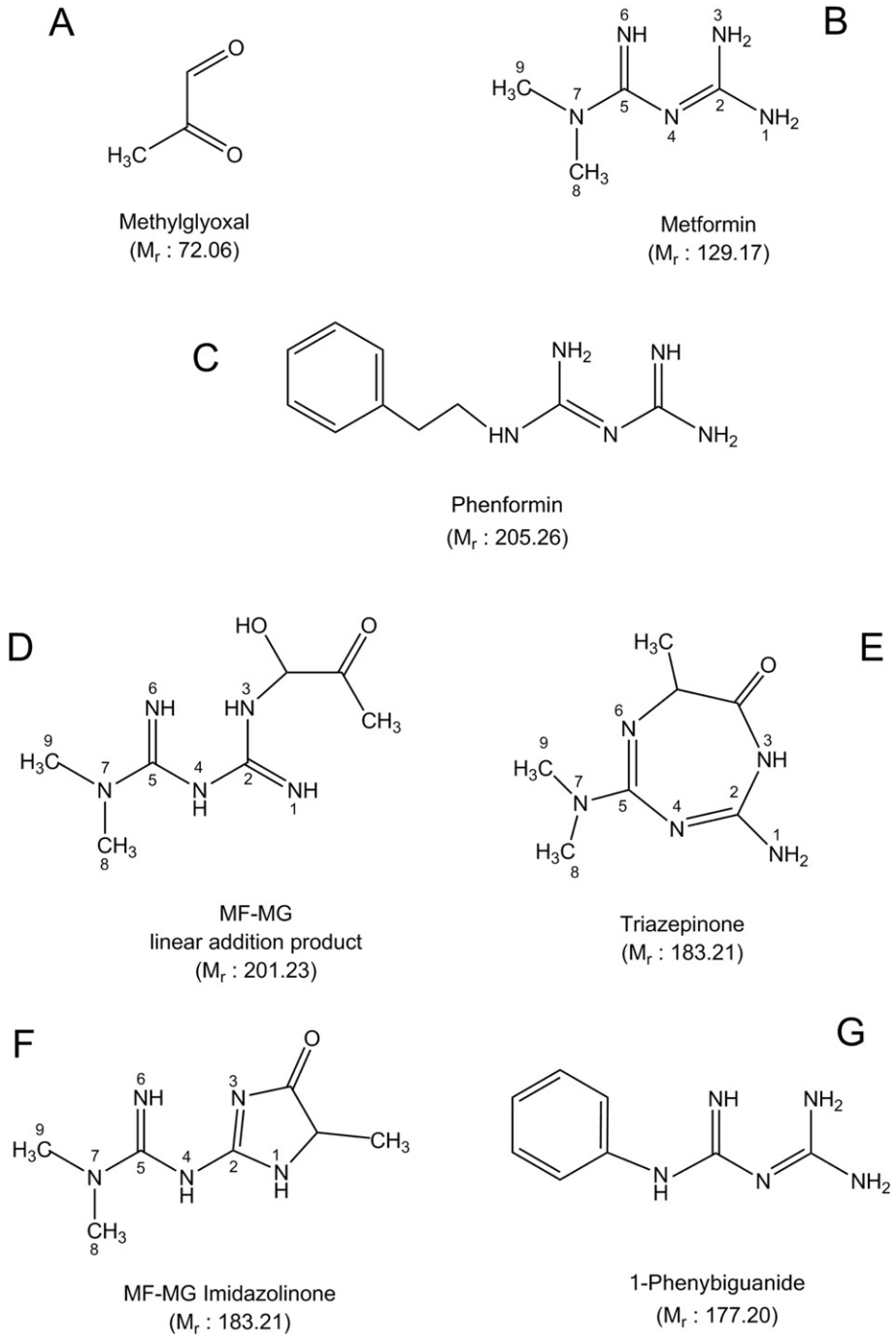

Fig. 1. Structure of methylglyoxal (MG), biguanide drugs metformin (MF), phenformin (PF) and 1-phenylbiguanide (PB), and the intermediates in the reaction of MF and MG.

pH suggests that the acid-base properties of biguanides could play an important role in the reaction mechanism.

2. Experimental

Metformin hydrochloride (*N*,*N*-dimethylimidodicarbonimidic diamide monohydrochloride), phenformin hydrochloride (*N*-(2-phenylethyl)imidodicarbonimidic diamide monohydrochloride), and 1-phenylbiguanide hydrochloride were purchased from Alfa Aesar, Fluka and Sigma Aldrich, respectively. All other salts, acids and buffers were of analytical grade quality and were used as received. Room-temperature ionic liquid (RTIL), i.e., tridodecylmethylammonium tetrakis[3,5-bis(trifluoromethyl) phenyl]borate (TDMATFPB), was prepared by the metathesis of the corresponding salts in acetone [10]. Aqueous electrolyte solutions were prepared from highly purified water with resistivity of 18.2 MΩ (Millipore).

CE/C^4D measurements were carried out using the Agilent 7100 Capillary Electrophoresis System (Agilent Technologies, Waldbronn, Germany) equipped with a contactless conductivity detector (C^4D), which was placed in the electrophoretic cassette thermostated at constant temperature of 25 °C. The C^4D had tubular electrodes 2.5 mm long with a 1.2 mm long detection gap between the electrodes and operated with a sine-wave signal with a frequency of 1.0 MHz and an effective voltage of 50 V [11]. CE separations were performed using an untreated fused-silica capillary (Composite Metal Services, UK), 25 μm id, with the total length of 31.5 cm (18.7 cm to C^4D), and the separation