ESSENTIAL DIFFERENCES IN SPONTANEOUS REACTION OF L-LYSINE AND L-ARGININE WITH FORMALDEHYDE AND ITS QUANTUM CHEMICAL INTERPRETATION

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Abstract

It has been recently reported by us that spontaneous reaction between formaldehyde and L-lysine yields N-methylated products at room temperature. In this paper we discussed this reaction extending to L-arginine. While the reaction between formaldehyde and L-lysine produces different N-methylated derivatives, no methylated only N^G-hydroxymethylated derivatives can be observed in the reaction mixture between formaldehyde and L-arginine. The difference in the reaction between L-lysine and L-arginine with formaldehyde can be explained with the different nucleophilicity of the amino group in lysine and that of the imino group in arginine examined by the electrostatic potential of N atoms by the ELPO quantumchemical calculation method of Náray-Szabó.

Introduction

It has been supposed for a long time that methylation of amines by formaldehyde takes place only under extreme conditions [1—4]. Means and Feeney [5] described later a method, using sodium borohydride, by which lysine side chains of proteins, or poly-lysine could be methylated with formaldehyde. Based upon Sörensen's [6] reaction it has been generally accepted that the reaction of formaldehyde with amino acids yields unstable N-hydroxymethyl derivatives which decompose easily in acidic medium [7—8]. This view seemed to be confirmed by theoretical quantumchemical calculation of Lipscomb and co-workers [9—10].

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Recently we have reported on the spontaneous reaction between formaldehyde and L-lysine yielding N-methylated products at room temperature [11, 12]. This reaction, extended to L-arginine as well, is discussed in the present paper. A tentative theoretical explanation of the experimental findings is outlined.

Experimental and theoretical

Materials — All materials were of analytical purity. L-Lys, L-Arg and N^{ε}-formyl-L-Lys came from Reanal, Budapest; D-Arg from Serva, N^{ε}-monomethyl-L-Lys from Sigma, $N^{\varepsilon},N^{\varepsilon}$-dimethyl-L-Lys from Vega Biochemicals, $N^{\varepsilon},N^{\varepsilon},N^{\varepsilon}$-trimethyl-L-Lys, N^{G}-monomethyl-L-Arg, N^{G},N^{G}-dimethyl-L-Arg and N^{G},N^{G}-dimethyl-L-Arg from Calbiochem while N^{G},N^{G},N^{G}-trimethyl-L-Arg flavionate was obtained from the Institute of Drug Research, Budapest.

Instruments — Potentiometric titration using combined glas electrodes with a RADELKIS OP-205 equipment. UV, ^{1}H NMR and mass spectra were recorded by a SPECORD UV-VIS (Zeiss) spectrophotometer, by a FX-JEOL instrument at 100 MHz in D_2O and by a JEOL O1 GS 2 mass spectrometer, respectively. FIXION 50×8 strong cationic exchange plates were used in a citrate buffer at pH = 6.1 for thin layer chromatography.

Methylation reaction — 100 cm^3 0.01M L-Lys and L-Arg were titrated potentiometrically at 25 °C by 0.03% formaldehyde solution which has been neutralized previously by 0.01N sodium hydroxide. Simultaneously with the titration samples of 10 mm^3 were chromatographically analyzed to detect the formation of the ninhydrine positive product during the next 24 hours. Kinetic measurements were performed by difference UV-spectrophotometry at pH = 7.34 and at room temperature. Reaction mixtures contained excess formaldehyde in a 0.5% concentration. The reference sample contained 0.5 mol/cm^3 arginine which was compared with a 1:1 mixture of 1.0 mol/cm^3 arginine and 1.0% formaldehyde solution. Arginine was applied in four initial concentrations: 0.25, 0.5, 0.75 and 1.0 mol/cm^3, respectively.

Preparation of N^{G}-hydroxymethyl-L-arginines

3 mM L-arginine was dissolved in a formaldehyde solution, diluted to 10% by 4.0 cm^3 phosphate buffer (pH = 7.34). This mixture was treated in an isolated system for 24 hours at 37 °C. 3.0 cm^3 of the obtained mixture was taken to a 1.8×65 cm Sephadex G-15 column equalized with distilled water. Fractions of 10 cm^3 were collected and analyzed spectrophotometrically. Spectrally positive fractions appeared from 70 cm^3 eluation volume indicating

that products with molecular weight larger than arginine have left the column. Arginine and formaldehyde standards were eluated in the 13th and 17th fraction respectively. Fractions from seventh to twelfth were unified and dried at room temperature yielding a white, crystalline mixture of isomers of N^G-hydroxymethyl-L-arginine. M. p. 189—194 °C. L-Arginine. $2CH_2O$ (calc.) C: 40.60, H: 7.69, N: 23.72; (found): C: 41.80, H: 7.90, N: 23.29.

Preparation of labeled N^G-hydroxymethyl DL-arginine (6—14 C)

0,17 g (1 mM) D-L-arginine (6—14 C) was dissolved in 15 ml distilled water has been reacted with 36% concentrated formaldehyde in cold condition for 48 hours. The reaction product was precipitated with 150 ml anhydrous acetone and was put to refrigerator and kept there 5 hours. After passing the time, acetone was deconted and the still hydrous hydroxymethyl derivative crystallines were scuffed again with 150 ml anhydrous acetone.

The crystallines were filtered, washed with acetone and dried in a vacuum exsiccator. Its specific radioactivity was determined with a liquid scintillated spectrometer (type: Berthold BF 5000). The content of the methylol-arginine derivative was examined by a radiochromatographic method (Previously described [11, 12] thinlayer chromatographic system). On the chromatogram (Fig. 1) could be seen four peaks. The specific activity of the methylol-arginine derivate: 11,41 mCi/mM (0,065 mCi/mg).

Stability studies—(a) Samples, containing 10 mg crystalline N^G-hydroxymethyl-L-arginines were dissolved in 2 cm^3 1N HCl, 0.1N HCl and 0.01N HCl, respectively. These solutions were held at 37 °C for 5 hours. (b) 10 mg crystalline N^G-hydroxymethyl-L-arginines was dissolved in 5 cm^3 0.4% dimedon solution and it was held at 37 °C for 5 hours. The same procedure was repeated at 100 °C for 1 hour.

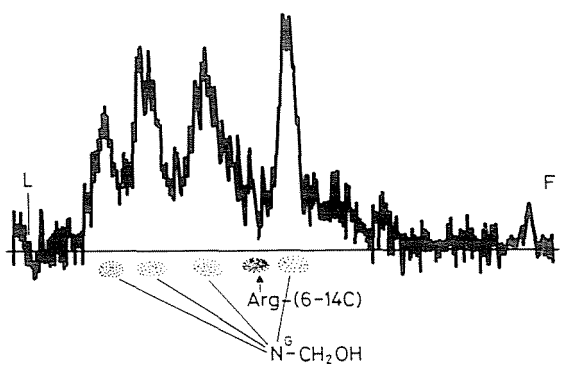


Fig. 1. Radiogram of N^G-hydroxymethyl L-arginines (6—14 °C) on FIXION 50 × 8 cation exchanging chromatoplate

Quantum chemical calculations — Electrostatic isopotential maps were calculated by the ELPO programme [13, 14]. Geometries of lysine [15] and arginine [16] were taken from neutron diffraction studies.

$$V(r) = \sum_{a=1}^{M} \frac{Z_a}{|R_a - r|} - V_e(r)$$

$$V_e(r) = \int \frac{\rho(r'_1)}{|r - r'|} \, dr'$$

where: V is the electrostatic potential at the point r, V_e detones the potential due to the electrones, while Z_a is the charge of the ath molecule located at R_a. M is the total number of atoms in the molecule, p is the charge density.

Results

Titration curves, combined with thin layer chromatograms for L-lysine and L-arginine are depicted in Figs 2 and 3. Changes in pH for both compounds correspond to the Sörensen formule titration [6]. Essential differences are seen in the figures. While L-lysine reacts slowly, its methylated products appear only 5 hours after neutralization, N^G-hydroxymethyl derivatives of arginine can be detected instantaneously on the plate. Methylation reaction products for L-lysine could be identified as N-mono-, di- and trimethyl-L-lysine (Fig. 2). No hydroxymethyl derivatives could be detected at all. On the other hand, reaction between L-arginine and formaldehyde is very fast, the products could be identified as N^G-hydroxymethyl derivatives (Fig. 3), with thinlayer chromatography.

Time dependence of the difference UV spectrum for the reaction mixture of L-arginine is shown in Fig. 4 while Table I displays first order reaction rate constants for L- and D-arginine and N^G-methylated products. NMR spectra of pure L-arginine and of its N^G-hydroxymethyl products shown in Figs 5/a, 5/b, verifying the N^G-hydroxymethyl structure (ppm 4,1—4,6, four singlet peaks on Fig. 5/b).

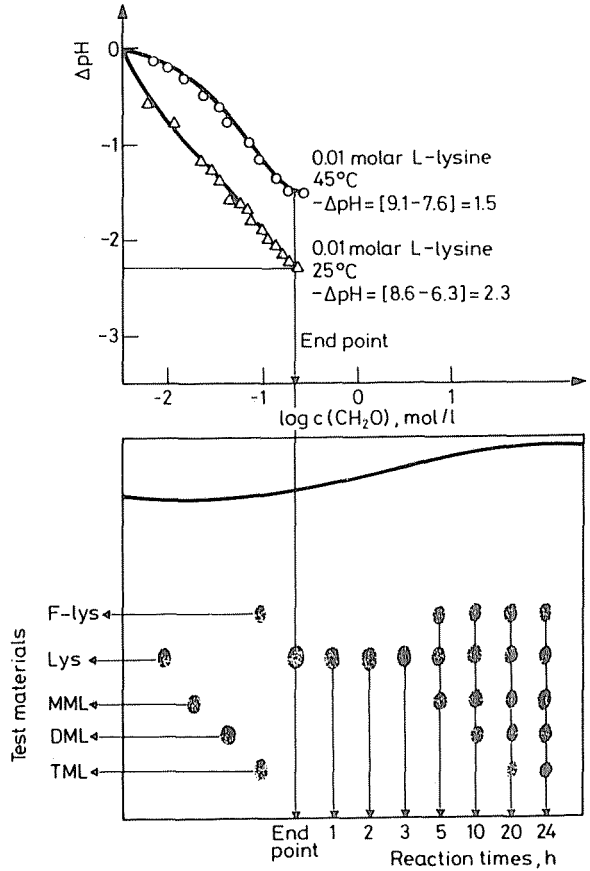


Fig. 2. Changes in pH values of 0,01 mol L-lysine solutions due to the effect of formaldehyde and the ninhydrine positive spots of lysine derivatives formed in the reaction

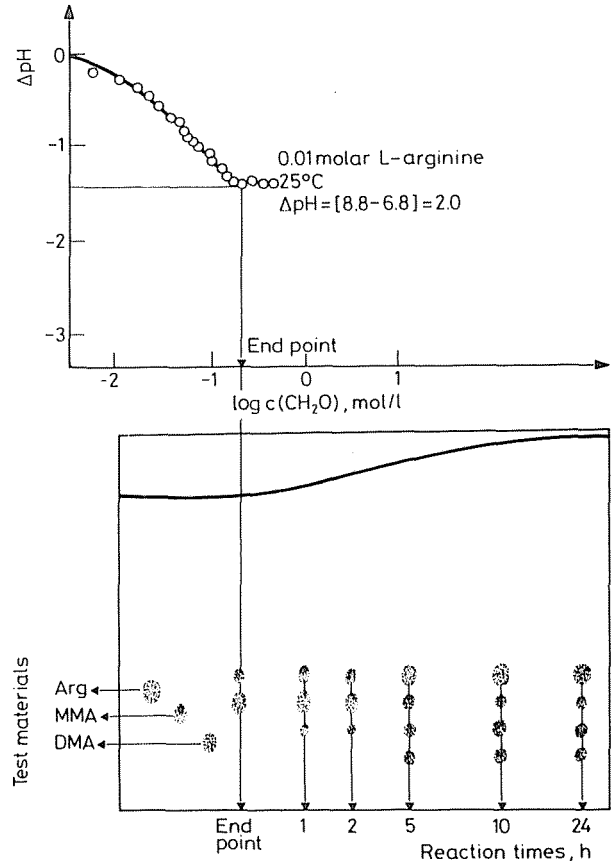


Fig. 3. Changes in pH values of 0,01 mol L-arginine solution due to the effect of formaldehyde and the ninhydrine positive spots of arginine derivatives formed in the reaction

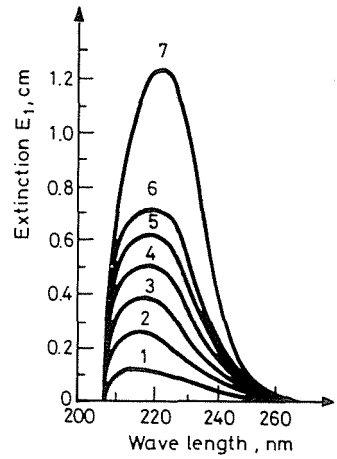


Fig. 4. Spectral tracking of the formation of methylol-arginine. Conditions: room temperature, pH = 7.38, concentration of arginine = 0,5 mmol/1. Specord UV-VIS (Zeiss) type spectrophotometer. 1 : 4 min, 2 : 8 min, 3 : 12 min, 4 : 16 min, 5 : 20 min, 6 : 24 min, 7 : 60 min

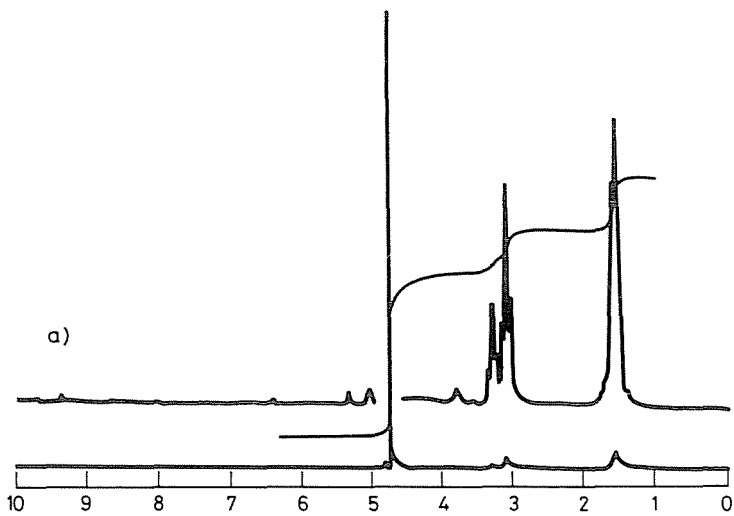


Fig. 5/a. ^{1}H NMR spectrum of L-arginine

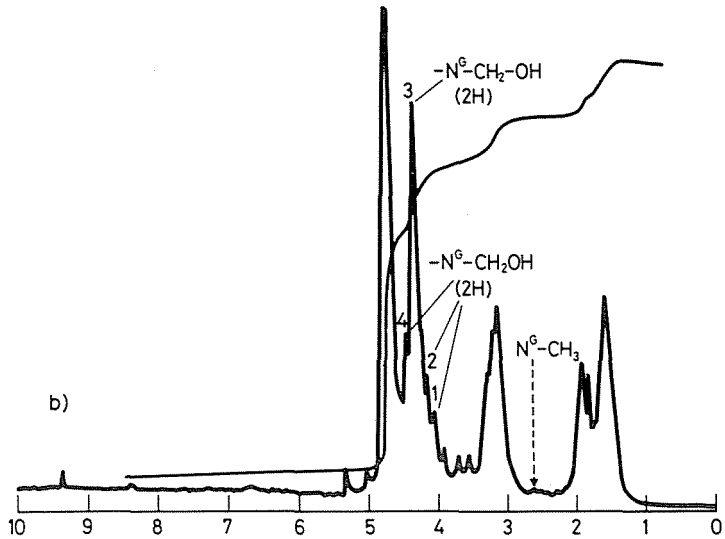


Fig. 5/b. ^{1}H NMR spectrum of N^G-hydroxymethyl arginines

Discussion

Two problems arise when studying the reaction between formaldehyde and L-arginine:

(1) what are the products formed;

(2) how the essential difference between reactivities of L-lysine and L-arginine can be explained.

It is evident from Table I that if the imino nitrogen of the arginine guanidino moiety is blocked by methyl groups the reaction gets practically impossible. On the other hand, N^G-monomethyl and N^GN^G-dimethyl derivatives of arginine react with formaldehyde at about the same rate. Consequently it is the imino nitrogen atom which reacts readily with formaldehyde to yield the monosubstituted hydroxymethyl derivative. Most probably the second formaldehyde molecule enters at the terminal amino nitrogen atom. This is understood by inspection of the electrostatic isopotential map of the zwitterionic, N-protonated form of L-arginine (Fig. 6). It is known, that the electrostatic potential around a given atom may serve as a measure of its nucleophilicity [17]. Consequently, Fig. 6 indicates that nucleophilic attack of the imino nitrogen at the carbon atom of formaldehyde is much more probable than of the amino group, since the potential is much lower in the vicinity of the former.

Table I

First order rate constants (min^{-1}) for the reaction between formaldehyde and selected compounds at 20°C

Compound	k
L-lysine	≈0
L-arginine	28.6
D-arginine	28.6
N^G-methyl-L-arginine	12.9
N^GN^G-dimethyl-L-arginine	10.9
N^G, N'^G-dimethyl-L-arginine	0.42
N^G, N^G, N'^G-trimethyl-L-arginine	0

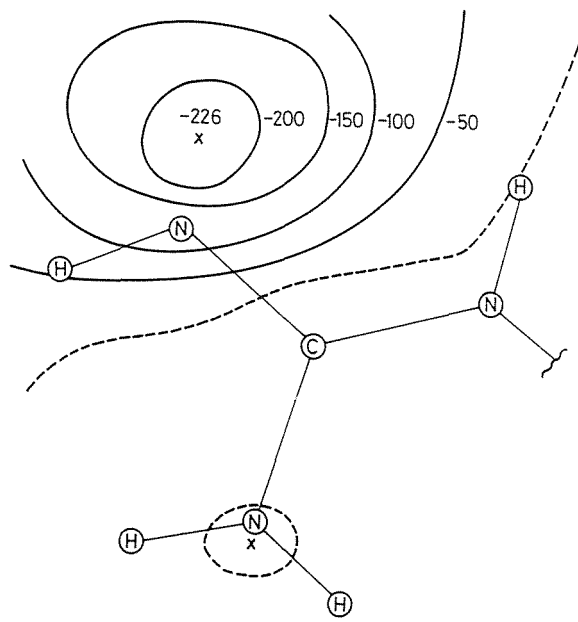
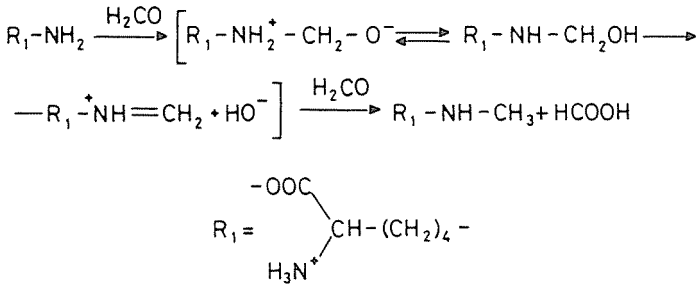


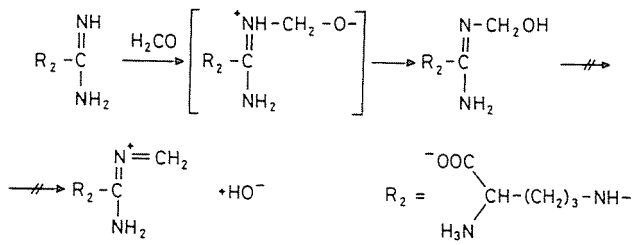
Fig. 6. Electrostatic isopotential map in a plane parallel to the guanidine group of L-arginine at a distance of 1,5 A. Dashed lines correspond to zero potential figures are given in kJ/mol

Let us compare now reactivities of L-lysine and L-arginine. In the case of lysine the reaction route may be the following (Scheme I): The N-protonated form of lysine with formaldehyde to yield an unstable adduct, afterwards a proton is transformed to the alkoxide group and hydroxymethyl-L-lysine is formed. This reacts at once with a second molecule of formaldehyde and the intermediate product is reduced via an azomethine structure to N-methyl-L-lysine.



Similar reactions were described by Sanders et al. [18] in a reductive methylation reaction with formaldehyde and $NaBH_4$, where the hydride aninon ($H^{\ominus}$) donor was $NaBH_4$. In our reaction the $H^{\ominus}$ donor is itself the formaldehyde molecule.

The same mechanism for L-arginine is outlined in Scheme II. The reaction ends at the hydroxymethylated product since formation of the methylene-iminium group in the reduction step is unfavoured. This is the reason why no methylated products of L-arginine can be detected during reaction. The difference in the reaction rate between L-lysine and L-arginine with formaldehyde (Table I) is explained with the different nucleophilicity of the amino group in lysine and that of the imino group in arginine. The minima of the electrostatic potential around the corresponding nitrogen atoms are -481 kJ/mol and -749 kJ/mol, respectively. These values explain the higher affinity of arginine, as related to lysine, towards the formaldehyde molecule.



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