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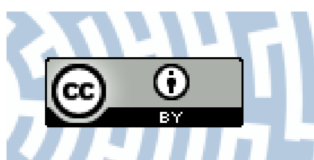

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Short Communication

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Circadian Rhythm of Spontaneous non-Linear Peptidization with Proteinogenic Amino Acids in Abiotic Solutions *versus* Homochirality

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Summary. In this short communication, we report on three striking phenomena of the circadian rhythm. One was observed with the non-linear concentration changes of the monomeric *L*-Cys and the non-linear yields of the *L*-Cys derived peptides, when undergoing spontaneous non-linear peptidization. The other one was observed with the binary *L*-Phe-*L*-Pro system, and the third one with *L*-Ser, *D*-Ser, and *DL*-Ser. So far, no analogous reports have been released on the circadian rhythm of the spontaneous non-linear peptidization of proteinogenic amino acids in a sterile abiotic environment (70% aqueous acetonitrile, or 70% aqueous methanol solutions). At the moment, we cannot find any rational explanation of this phenomenon, yet it seems highly probable that its origin is analogous to or even of a primordial nature for the circadian rhythm phenomena abundantly found in biological samples by other researchers. An experimentally established lack of the circadian rhythm with peptidization of the non-proteinogenic amino acid (*D*-Ser) can encourage us to revisit a still unsolved question of homochirality preconditions.

Key Words: circadian rhythm, proteinogenic amino acids, spontaneous non-linear peptidization, HPLC-ELSD, turbidimetry, homochirality precondition

Experiment

Spontaneous non-linear peptidization of proteinogenic amino acids dissolved in aqueous organic solvents was demonstrated in our earlier reports (e.g., [1–5]), and possible molecular mechanism of this process was also discussed. These investigations were carried out for the solutions containing single amino acids and the amino acid pairs. In paper [5], a theoretical model was proposed assuming four possible cases of spontaneous peptidization in solutions containing a pair of amino acids.

The main analytical tool employed in the aforementioned papers to trace spontaneous non-linear changes of the amino acid concentration in the course of sample storage (and ageing) was high-performance liquid chro-

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matography with evaporative light scattering detection (HPLC–ELSD). With its aid, the monomeric amino acid fraction can be separated from the spontaneously formed peptides and the time series of the changing peak heights valid for the monomeric amino acid witnesses to its non-linear concentration changes in the course of storage. These changes provide an indirect hint as to the non-linear changes of the resulting peptide yields also. Direct evidence of the non-linear changes of peptide yields can be obtained with aid of turbidimetric measurements in continuous mode. Such measurements can reveal non-linear turbidity changes of the amino acid solution (unequivocal with the changing peptide yields) right from the moment of the amino acid dissolution and long before the insoluble higher peptides precipitate, which then can be traced with human eye.

Currently, we intend to focus on our results mostly published elsewhere, yet without earlier pointing out to one of the most striking features of the spontaneous non-linear peptidization of the proteinogenic amino acids, and namely on the circadian rhythm of this process, revealed both with aid of HPLC-ELSD and turbidimetry.

First example demonstrates the circadian rhythm of the non-linear concentration and the yield changes with the monomeric *L*-Cys and the *L*-Cys-derived peptides, respectively, as traced in the course of the 4 days lasting *L*-Cys solution storage. Details concerning the experimental conditions are given elsewhere [6] and here, let us only emphasize that the investigated samples were protected from the day-night light changes (both in the autosampler of the chromatograph and the turbidimetric cell). Moreover, the chromatographic column of the C_{18} type used for the HPLC-ELSD experiments was thermostatted at 35°C, and turbidimeter was put in a foamed polystyrene box to preserve constant temperature throughout an entire experiment (22±0.5°C). Last not least, the *L*-Cys sample was prepared in the aseptic 70% aqueous acetonitrile. The results obtained are shown in *Fig 1(a),(b).* Above each of the two plots, roughly estimated periods of the plot shape repetitions are given. In the case of the concentration changes with the monomeric *L*-Cys, these periods range from 23.75 to 24.95 h, and the analogous repetition periods on the plot of the peptide yield changes range from 21.60 to 25.20 h. Taking into account the physical difference between the monomeric *L*-Cys concentration and an overall turbidity of the peptides containing *L*-Cys solution, and the difference in accuracy of the chromatographic and turbidimetric measurements, there is no doubt that the two result sets given in *Fig. 1(a),(b)* unequivocally witness to the circadian rhythm of the *L*-Cys peptidization.

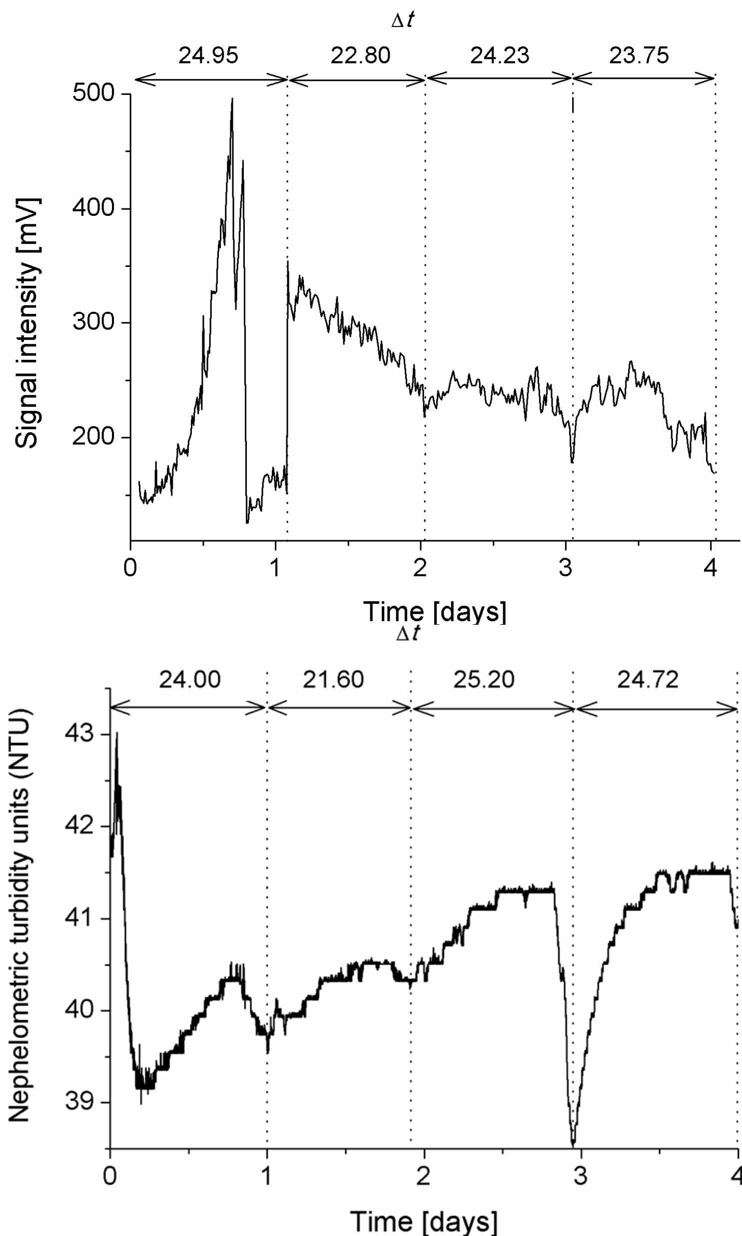

Fig. 1. (a) The chromatographic peak height changes of the monomeric *L*-Cys and (b) the turbidity changes for the *L*-Cys solution in 70% aqueous acetonitrile in the initial 4 days of the sample storage. Concentration of *L*-Cys was equal to 0.7 mg mL^{-1} (5.77×10^{-3} mol L^{-1}). Duration of the plot shape repetition periods (Δt, [h]) is marked above the respective plots

Second example demonstrates the circadian rhythm of the non-linear concentration changes with *L*-Phe and *L*-Pro in the binary *L*-Phe-*L*-Pro system, and the circadian rhythm of the turbidity changes in the same system, as traced in the course of the 11 days lasting sample storage. The HPLC-ELSD results valid for second example were originally presented in paper [3]. The *L*-Phe-*L*-Pro sample was also dissolved in 70% aqueous acetonitrile, and it was protected from the day-night light changes in the autosampler of the chromatograph and the turbidimetric cell, respectively. The chromatographic column of the C_{18} type was thermostatted at 35° and the turbidimeter temperature was kept steady in the previously described way (22±0.5°C). The results obtained are shown in *Fig. 2(a),(b).* Periodicity of the plot shape repetitions (HPLC-ELSD) valid for *L*-Phe and *L*-Pro ranges from 18.23 to 25.67 h, yet in most cases the repeatability periods are closer to each other and range from 20.40 to 23.51 h *(Fig. 2(a)).* Even more regular is the shape of the registered turbidity plot *(Fig. 2(b))* and in this case, the plot repeatability periods hold between 19.66 and 26.80 h. Thus it can be stated that spontaneous peptidization of *L*-Phe and *L*-Pro in the binary *L*-Phe-*L*-Pro system proceeds in the circadian rhythm.

(a)

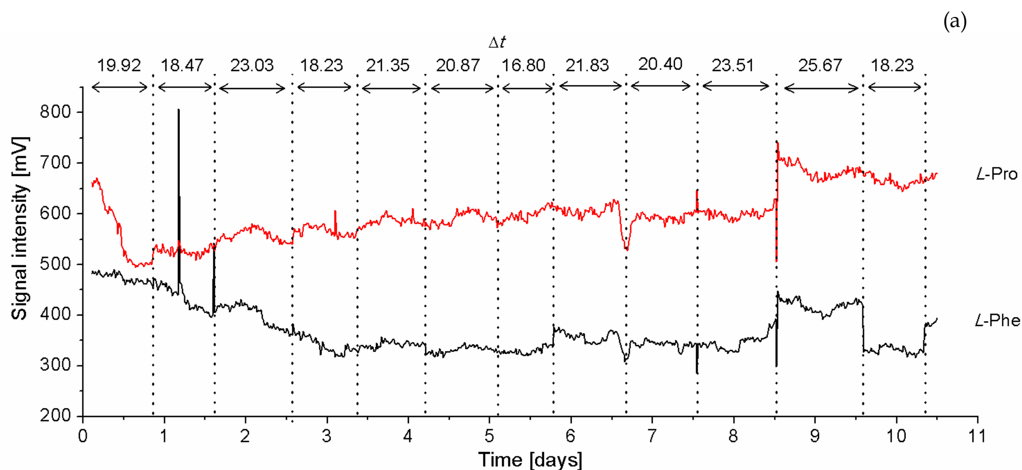

Fig. 2. (a) The chromatographic peak height changes of the monomeric *L*-Phe and *L*-Pro in the binary *L*-Phe-*L*-Pro solution in 70% aqueous acetonitrile and (b) the turbidity changes for the *L*-Phe-*L*-Pro solution in 70% aqueous acetonitrile in the initial 11 days of sample storage. Concentration of *L*-Phe and *L*-Pro was equal to 1.0 and 1.0 mg mL^{-1} (6.05×10^{-3} and 8.69×10^{-3} mol L^{-1}), respectively. Duration of the plot shape repetition periods (Δ*t*, [h]) is marked above the respective plots

(b)

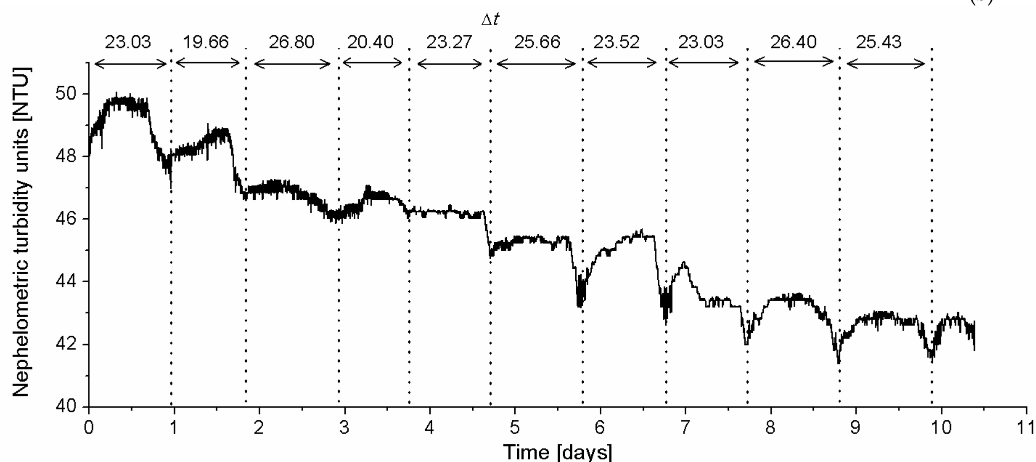

Fig. 2. Cont.

The turbidimetric results of the third experiment are presented for the first time now and they refer to *L*-Ser (proteinogenic amino acid), *D*-Ser (non-proteinogenic amino acid), and *DL*-Ser (the racemic mixture of the Ser enantiomers) dissolved in a strongly antiseptic 70% aqueous methanol. These results were recorded in the course of the 7 days sample storage period and similar to the two aforementioned examples, also in this case the turbidimeter was kept at constant temperature (22±0.5°C) throughout an entire experiment. The results obtained are given in *Fig. 3(a)–(c)*. Based on the plot presented in *Fig. 3a* and valid for *L*-Ser, it can be stated that although the plot shape repetition periods differ from 19.66 to 32.63 h, most of them range from 21.12 to 25.20 h, which well corresponds with the circadian rhythm. In the case of *D*-Ser (which is a non-proteinogenic amino acid), no circadian peptidization rhythm is observed and sample turbidity is monotonously growing (in pace with the growing peptide yields), except for one maximum after ca. 2 days storage period *(Fig. 3(b))*. In the case of *DL*-Ser, irregular repetitions of the plot shape pattern are observed, apparently as a kind of a 'compromise' between the regularity of the *L*-Ser pattern and no plot shape repetitions with *D*-Ser *(Fig. 3(c))*. The most amazing outcome of this experiment is that the circadian peptidization rhythm holds for the proteinogenic *L*-Ser only, whereas no such rhythm is observed with the non-proteinogenic *D*-Ser. Thus a supposal can be formulated that maybe the necessary precondition for the homochirality of amino acids in human and

animal tissues is founded on the circadian rhythm of the proteinogenic amino acids involved, although a tedious and thorough experimental confirmation of this supposal is inevitable.

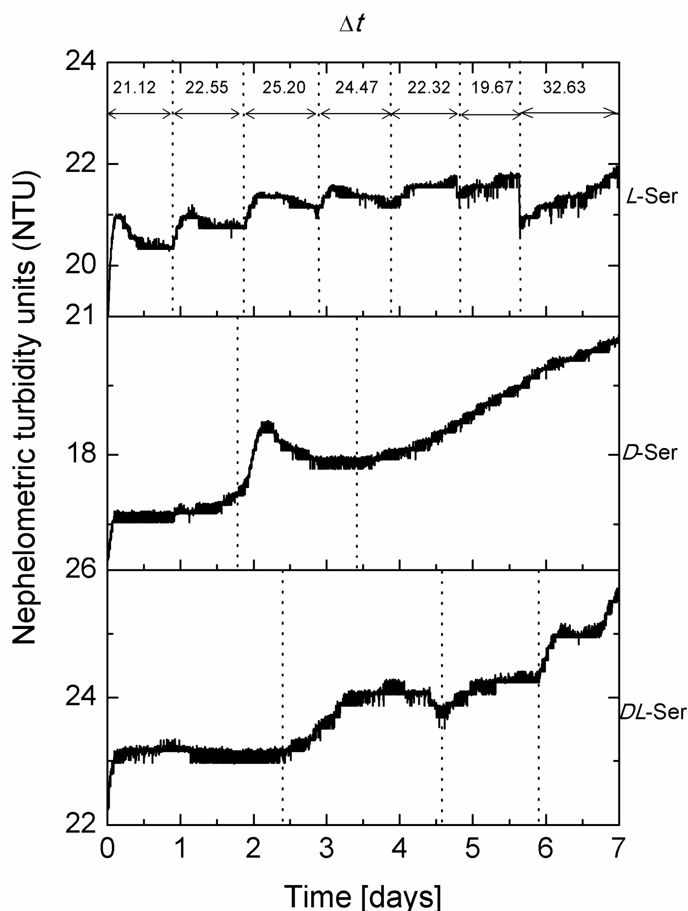

Fig. 3. The turbidity changes for the *L*-Ser, *D*-Ser, and *DL*-Ser solution in 70% aqueous methanol in the initial 7 days of sample storage. Concentration of *L*-Ser, *D*-Ser, and *DL*-Ser was equal to 1.0 mg mL^{-1} (9.44×10^{-3} mol L^{-1}). Duration of the plot shape repetition periods (Δt, [h]) valid for *L*-Ser is marked above the plot of *L*-Ser

Finally, it has to be added that the turbidimetric measurements were performed for all solvents used in our experiments (water, acetronitrile, methanol, 70% aqueous acetonitile, and 70% aqueous methanol), and stability of the turbidity levels was confirmed with each tested solvent system.

Discussion

The circadian rhythm of the peptide concentration changes and the other physiological phenomena has been revealed by many researchers in biological material, and investigated both under the *in vivo* and *in vitro* conditions (e.g., [8–13]). Up to our best knowledge, this is the first report in the literature on the circadian rhythm of the non-linear concentration changes with the monomeric amino acids and the resulting peptides, carried out in full abstraction from any biological matter, and with use of the commercially obtained amino acids of analytical purity and the HPLC purity grade solvents only.

Conclusion

We cannot offer any rational explanation of the circadian rhythm observed with four proteinogenic amino acids discussed in this study (and not taking place with one non-proteinogenic amino acid). It can only be speculated that the proteinogenic amino acids formed in the evolutionary course of biogenesis characterize with physicochemical properties (like, e.g., the pK_a values, the dipole moments, the electric permeabilities, etc.) which in some way promote this circadian rhythm. Moreover, it seems probable that the circadian rhythm of spontaneous non-linear peptidization observed with proteinogenic amino acids is of a primordial nature and the same mechanism is responsible for the circadian rhythms widely observed with biological matter. In other words, maybe the proteinogenic amino acids act as triggers of the circadian clocks abundantly observed in Nature. Last not least, an observation of the circadian peptidization rhythm with *L*-Ser and no such effect with *D*-Ser naturally reminds us of the so far unknown sources of homochirality. In future experiments, the dynamics of spontaneous peptidization with *D*-isomers of proteinogenic amino acids should be tested to find out if the circadian peptidization rhythm occurs with the proteinogenic *L*-amino acids only.

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