Impact of $D_2O$ on peptidization of $L$-histidine

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Received: 18 April 2021 / Accepted: 23 May 2021 / Published online: 28 May 2021
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Abstract
This is our sixth consecutive study carried out in an order to collect an experimental evidence on the impact of heavy water ($D_2O$) on spontaneous peptidization of the proteinogenic $\alpha$-amino acids and this time it is $L$-histidine ($L$-His). Scientists have not yet achieved a full consensus regarding the source of this very important amino acid in human and mammalian tissues, and on this particular question rather contradictory answers in form of experimental results are produced, equally supporting its exogenous and endogenous origin. Although this issue still remains unsolved, for practical demands of life sciences the two UN agencies, FAO and WHO, have both tentatively accepted that $L$-His is an exogenous $\alpha$-amino acid. As analytical techniques, in our studies we employed high-performance liquid chromatography with the diode array detection (HPLC–DAD), mass spectrometry (MS), and scanning electron microscopy (SEM). Spontaneous peptidization of $L$-His dissolved in methanol + $H_2O$, 7:3 ($v/v$) was carried out at $22 \pm 0.5 \, ^\circ C$ in the darkness for a relatively long period of 314 h, and its progress was chromatographically checked by targeting concentration of the $L$-His monomer in the 12-min intervals. This investigation revealed alternating yet non-periodic concentration changes, indicating changeable formation and hydrolytic decay of the $L$-His-derived oligopeptides in the function of time, and a fast net concentration fall of the $L$-His monomer (witnessing to quite vigorous peptidization). Moreover, the MS results confirmed formation of the relatively high oligopeptides, falling within the range of two or more dozen $L$-His monomer units. Impact of $D_2O$ on peptidization of $L$-His was traced with use of MS and SEM for the $L$-His samples dissolved in aqueous methanol solvents containing 5, 10, 20, and 30% $D_2O$, and also in pure $D_2O$. Similar to the results earlier presented for five other proteinogenic $\alpha$-amino acids, heavy water exerts a powerful inhibitory effect on spontaneous peptidization of $L$-His, equally perceptible when assessed with aid of mass spectrometry (with the mass spectra in the first instance playing the role of quasi-quantitative fingerprints), and based on purely qualitative micrographs derived with use of SEM.

Keywords $L$-histidine · $D_2O$ · Spontaneous peptidization · High-performance liquid chromatography · Mass spectrometry · Scanning electron microscopy
Introduction

This paper is the sixth one in a series devoted to investigation of the impact of heavy water (D_2O) on spontaneous oscillatory, or fluctuating peptidization of proteinogenic α-amino acids and it focuses on L-histidine (L-His). Three earlier reports have focused on the impact of D_2O on spontaneous oscillatory peptidization of L-cysteine (L-Cys) [1], L-proline (L-Pro) [2], and L-alanine (L-Ala) [3], and on spontaneous fluctuating peptidization of L-methionine (L-Met) [4] and L-hydroxyproline (L-Hyp) [5]. The most important findings of these studies were summarized in a mini-review [6]. The starting point with each paper from the series [1–5] demonstrated that if α-amino acid was dissolved in the aqueous-organic solvent (yet in an absence of D_2O), it underwent spontaneous oscillatory or fluctuating peptidization (as experimentally shown with aid of the non-chiral high-performance liquid chromatography, HPLC, and mass spectrometry, MS). Moreover, this phenomenon had been inaugurated by and then has run in the parallel with another spontaneous oscillatory process, which was chiral inversion of the investigated α-amino acids. In fact, it has been demonstrated in a series of papers that chiral inversion was inherent not only of α-amino acids, but of a variety of other chiral derivatives of acetic and butyric acid (as documented, e.g., in papers [7–13]).

First experimental examples of spontaneous oscillatory chiral inversion of the low molecular weight carboxylic acids given in our reports encouraged an onset of theoretical models of oscillatory phenomena related to chirality [14]. In a similar way, first experimental demonstrations of spontaneous oscillatory condensation gave rise to an onset of theoretical models, in the first instance focused on peptidization of α-amino acids as the most important building blocks of all living matter [15–18]. It was also revealed that peptidization rhythm of two endogenous species (L-Cys and L-Pro) was circadian, yet no such regularity was observed with the two exogenous species (L-Met and L-Hyp) [6]. The most recent and quite spectacular theoretical models of spontaneous oscillatory peptidization able to result in formation of trimeric, tetrameric and pentameric oligopeptides are introduced in paper [19].

An impact of D_2O on spontaneous oscillatory or fluctuating peptidization of proteinogenic α-amino acids [1–6] can briefly be characterized as inhibition of this process, no matter whether it has been tested upon endogenous (L-Cys, L-Pro, and L-Ala), or exogenous (L-Met, L-Hyp) species. If pure D_2O was used as a solvent, almost complete extinction of the alternating peptidization mechanism with all so far investigated species was observed. When, however, different organic–inorganic solvents, i.e., MeOH + X (or ACN + X), 70:30 (v/v) were used (where X: mixture of H_2O and D_2O, with volume fraction of D_2O changing between 0 and 0.30), an observed inhibitory effect of heavy water was incomplete and not the same with all the investigated species, and its dynamics largely depended on molecular structure of a given compound. It was also observed that—independent of the content of D_2O in solution—its inhibitory effect was stronger pronounced against formation of higher peptides than the lower ones (which seems rather understandable, as peptidization route towards higher peptides leads via the lower ones).
In this study, we are going to examine an impact of D$_2$O on spontaneous fluctuating peptidization of L-His, which in physiological terms is a very interesting species, and for the following reason: It is synthesized in the organisms of humans (and mammals), although in the case of humans at least, in seemingly insufficient amounts and for this reason, it is routinely supplemented with normal diet. Numerous experiments carried out with the short-term [20, 21] and long-term L-His-depleted diet [22–24] did not show an impact of its lack on the nitrogen balance of the organism, most probably due to the maintenance of large L-His pools in the organism in the form of hemoglobin and carnosine. In the meantime, occasional reports have been published either claiming an essential role of L-His [25], or otherwise [26]. In 1985, despite a controversy regarding its essentiality L-His was accepted by the Food and Agriculture Organization (FAO) and World Health Organization (WHO), both the United Nations agencies, as an indispensable amino acid [27]. Further investigations carried out beyond that date left an issue of essentiality (or otherwise) of L-His still unresolved and open [28], yet currently L-His is formally accepted as essential [29].

**Experimental**

**Reagents and samples**

In our experiments, we used L-His of analytical purity, purchased from Reanal (Budapest, Hungary), methanol (MeOH) of HPLC purity (Merck KGaA, Darmstadt, Germany) and heavy water (D$_2$O) (Cambridge Isotopic Laboratories, Andover, MA, USA; 99% purity). Water (H$_2$O) was de-ionized and double distilled in our laboratory by means of the Elix Advantage model Millipore System (Molsheim, France). The L-His sample prepared for the HPLC–DAD experiment was dissolved at a concentration of 1 mg mL$^{-1}$ in MeOH + H$_2$O, 70:30 (v/v). All L-His solutions used for liquid chromatography (HPLC–DAD), mass spectrometry (MS) and scanning electron microscopy (SEM) were prepared at a concentration of 1 mg mL$^{-1}$ either in pure D$_2$O, or in the binary liquid mixtures MeOH + X, 70:30 (v/v), where X: the binary mixture of H$_2$O + D$_2$O in the changing volume proportions of 30:0, 25:5, 20:10, 10:20 and 0:30 (thus making a totality of the six test samples).

**High-performance liquid chromatography with diode array detection (HPLC–DAD)**

High-performance liquid chromatography with the diode-array detection (HPLC–DAD) was employed to separate the monomeric L-His from the peptides. The experiment was carried out for 314 h using a Varian model 920 liquid chromatograph equipped with a 900-LC autosampler, gradient pump, 330 DAD detector, and column thermostat (set at 35 °C) using a Varian Pro Star 510 column oven. The analyses were run for the 10-μL sample aliquots and a MeOH–water (90:10, v/v) mobile phase at a flow rate of 0.60 mL min$^{-1}$ with use of the ThermoQuest Hypersil C18 column (150×4.6 mm i.d.; 5 μm particle size). The Galaxie software was
used for data acquisition and processing. Relatively short sampling time intervals (12 min) were chosen in order to acquire quasi-kinetic information about spontaneous fluctuating peptidization. For the assessment of quantitative changes of the chromatographic signal valid for the monomeric L-His we used the results recorded at 222 nm.

**Mass spectrometry (MS)**

All mass spectra for the investigated L-His samples were recorded in the positive ionization mode with use of a Varian MS-100 mass spectrometer (extended ESI–MS scan, positive ionization, spray chamber temperature 50 °C, drying gas temperature 250 °C, drying gas pressure 25 psi, capillary voltage 50 V, and needle voltage 5 kV). Mass spectrometric detection was carried out straight away after a 7-day sample storage period. Samples were kept in the darkness at 25.0 ± 0.5 °C. Mass spectra were recorded for the solutions which contained the soluble peptide fraction (as the insoluble suspensions of microparticles self-separated by sedimentation).

**Scanning electron microscopy (SEM)**

The analyses performed within the framework of this study were carried out with use of a JEOL JSM-7600F model scanning electron microscope (SEM) for the six investigated L-His samples after two months storage period (at 22.0 ± 0.5 °C). Visualization of the nano- and microparticles was obtained for the respective solutions evaporated to dryness.

**Results and discussion**

The results presented in this paper are complemented with Supplementary material which contains schematic representation of the process of spontaneous chiral inversion of the L-His molecule (Fig. S1a), of its spontaneous peptidization (Fig. S1b), and of these two processes of chiral inversion and peptidization running in the parallel (Fig. S1c). Schemes given in Fig. S1 represent our current understanding of the molecular-level mechanisms of the aforementioned processes upon an example of L-His. In Fig. S2a, we present the chromatographic peak heights for the L-His monomer in MeOH + H2O, 70:30 (v/v), and in Fig. S2b, we give the power spectrum, calculated based on the time series of the chromatographic peak heights shown in Fig. S2a. In Fig. S3, we present a wider selection of micrographs which provide a more detailed illustration of the discussed peptidization process with L-His than barely four micrographs included in the main body of this paper. In the subsequent subsections, we discuss an impact of D2O on dynamics of peptidization with L-His, as it arises from the results obtained with use of three different instrumental techniques employed in this study.
High-performance liquid chromatography with diode array detection (HPLC–DAD)

The goal of storage and ageing of the L-His solution in MeOH + H₂O, 70:30 (v/v) was to demonstrate spontaneous and non-linear (i.e., fluctuating) nature of peptidization in an absence of heavy water, for further comparison of these results with those referring to ageing of L-His in the presence of D₂O. Spontaneous peptidization of L-His could only be demonstrated by separation of the L-His monomer from the spontaneously formed peptides and tracing its varying amounts, as represented by the changing peak heights of the L-His monomer (with changes resulting from the alternate processes of spontaneous peptidization and hydrolytic decay of the peptides). Storage of the L-His solution in MeOH + H₂O, 70:30 (v/v) was carried out for 314 h in the darkness at 22.0 ± 0.5 °C, and chromatographic control of the changing L-His monomer concentration was performed with use of the achiral HPLC–DAD system. We performed sampling of the L-His solution in the 12-min intervals, in order to gain a semi-kinetic insight in the dynamics of spontaneous peptidization of the discussed compound. In Fig. S2a representing the so-called time series (Supplementary material), we can see the changing L-His peak heights plotted against the sample storage time. Retention time of the monomeric L-His was equal to \( t_R = 5.53 \) min and the presented time series is based on chromatographic data recorded at the wavelength \( \lambda = 222 \) nm. Fig. S2a illustrates a non-linear (fluctuating) decay of the monomeric L-His (consumed at an expense of the oligopeptide formation), with the intensity of the starting signal at ca. 82 mAV and then its steep net drop to the final value of ca. 50 mAV.

To assess whether the HPLC signal recorded for the L-His monomer contains a significant periodic component, we performed the Fourier transform on the data shown in Fig. S2a and the power spectrum calculated for the L-His monomer peak is plotted in Fig. S2b. However, Fourier transform applied to this data set did not disclose periodicity within the investigated time series.

The experiment of storing L-His for 24 h in pure D₂O led to a virtually unchanged chromatographic peak height of this compound (within the experimental error limits below the 1% peak height), equivalent to the practically unchanged concentration of the monomeric L-His throughout an entire storage period. Based on this observation, we assumed that in contrast to the environment consisting of 70% aqueous MeOH, the 100% D₂O environment fully inhibits the process of spontaneous fluctuating peptidization of L-His.

Mass spectrometric (MS) tracing of spontaneous peptidization of L-His

Mass spectrometric technique allows registration of mass spectra of the L-His monomer and of spontaneously derived soluble peptides only. Mass spectra recorded for each individual L-His sample are given in Fig. 1. Prior to the registration, spontaneous peptidization process of all six L-His samples was carried out for 7 consecutive days in the darkness at 25 ± 0.5 °C and only after that time, we recorded for
each sample the mass spectrum in the \( m/z \) range from 100 to 3000 units, in order to expose a wide enough range of soluble peptides formed during the sample storage period. In the first instance, we regard these spectra as fingerprints illustrating an overall trend in formation of the soluble \( L \)-His-derived peptides and we make a comparison thereof in this section. However, we avoid ascribing individual signals to particular chemical structures as a rather futile task, due to very high numbers of signals in each individual spectrum and to a rather broad scope of structural possibilities in view. Nevertheless, certain signals can easily be ascribed to the \( L \)-His-derived peptides as direct evidence not only of their presence, but also of their complexity in the discussed solutions. For the sake of example, in solution containing 0% \( \text{D}_2\text{O} \), we ascribed signal at \( m/z \) 1800.75 to the oligopeptide built of thirteen \( L \)-His monomer units (\([\text{His}_{13}]^+\); Fig. 1a). In solution containing 20% \( \text{D}_2\text{O} \), we ascribed signal at \( m/z \) 2349.52 to the oligopeptide containing seventeen \( L \)-His monomer units (\([\text{His}_{17}]^+\); Fig. 1d). In solution containing 30% \( \text{D}_2\text{O} \), we ascribed signals at \( m/z \) 1662.40 and 2641.81 to the structures built of twelve (\([\text{His}_{12}-\text{H}]^+\)) and nineteen (\([\text{His}_{19}+\text{H}_2\text{O}]^+\)) \( L \)-His monomer units (Fig. 1e). Ascribing structures to a few selected MS signals makes it easily understandable that spontaneous peptidization of \( L \)-His results in oligopeptides of quite considerable molar weights, which fall in the range of two and perhaps even more dozen \( L \)-His monomer units. At the end, it needs to be added that the even higher and hence, insoluble peptides spontaneously separate from the solution by sedimentation and they are analyzed with use of the scanning electron microscopy (SEM), as it will be shown in the next section.

For the \( L \)-His solution which contains zero heavy water (\( \text{D}_2\text{O} \)), mass spectrum appears in the range of the signal intensity units up to 1200 kCounts (Fig. 1a), whereas for the remaining five samples (Fig. 1b–f), we give all mass spectra in a uniform signal intensity range up to 400 kCounts. A brief glance at all six mass spectra

\[ \text{Fig. 1} \ \text{Mass spectra recorded for } L-\text{His dissolved in MeOH+X, 70:30 (v/v), where X: the binary mixture of H}_2\text{O+D}_2\text{O in the changing volume proportions; a 0\% D}_2\text{O; b 5\% D}_2\text{O; c 10\% D}_2\text{O; d 20\% D}_2\text{O; e 30\% D}_2\text{O and f L-His dissolved in pure D}_2\text{O} \]
shown in Fig. 1 suffice to perceive how strong inhibitory effect heavy water exerts on peptidization of L-His, which is visible immediately from the lowest proportion of 5% D$_2$O. Apparently, the higher is the proportion of D$_2$O in solution, the stronger pronounced is its inhibitory effect on peptidization. In the case of sample dissolved in pure D$_2$O (Fig. 1f), the observed peptide yields are the lowest of all, and yet we cannot exclude that some trace amounts of the low-molecular-weight L-His-derived peptides result from technological process of the amino acid production (owing to an ability of all α-amino acids to instantaneously peptidize in aqueous-organic solvents, as documented in our earlier studies; e.g., in [1–5]).

In purely qualitative terms, the relationship between growing proportions of heavy water in solution and sinking yields of soluble lower peptides observed for L-His is analogical to the trends observed with L-Hyp [5] and L-Ala [3]. In the case of L-Cys [1], the amounts of lower soluble peptides are growing with growing proportions of D$_2$O at an expense of drastic inhibition of the higher insoluble peptides formation (as documented with use of the SEM technique). This trend characteristic of L-Cys is also observed with L-Met [4] and one can only speculate that this analogy has something to do with certain resemblance between L-Cys and L-Met, the only two proteinogenic α-amino acids which contain the sulphur atom. In the case of L-Pro, mass spectrometric results are still different [2]. With 5 and 10% D$_2$O, we observe lowering of the soluble peptide yields, whereas the 20 and 30% proportions reverse this trend toward the levels surpassing those observed for 5 and 10% D$_2$O. As expected, in pure heavy water the aliquots of the lower L-Pro derived peptides are negligible (as it is the case with the soluble lower peptides derived from the other α-amino acids discussed in this paragraph).

**Scanning electron microscopic (SEM) tracing of spontaneous peptidization of L-His**

From the mass spectra recorded for the L-His solutions with the growing proportions of D$_2$O, an unequivocal and strongly pronounced trend emerges of the step-wise lowering yields of the soluble L-His-derived peptides (Fig. 1), which is analogical to the results obtained with use of the scanning electron microscopy (SEM). Micrographs of the higher and mostly insoluble peptides derived from L-His show firm and rapid decrease in the yields with the increasing amounts of D$_2$O in solution. For each sample, we took a series of micrographs at different magnifications and from different sample locations on the test pin, and in Figs. 2 and S3, we gave selected micrographs which illustrate the observed trends. In Figs. 2a and S3a, we present micrographs recorded for the L-His sample which underwent peptidization in an absence of D$_2$O and the resulting peptide structures resemble flat, densely packed and irregularly shaped tiles. Addition of 5% D$_2$O dramatically changes pattern of the resulting peptide structures, which now become dispersed and irregular clumps with dimensions below 15 μm (Fig. S3b). The same trend extends to 10% D$_2$O in solution (Figs. 2b and S3c), with even smaller and more dispersed clumps of an average size of ca. 10 μm and less. Gradual rise of the D$_2$O contents to 20, 30, and 100% intensifies the same trend, with even more dispersed and even smaller
peptide clumps (Figs. 2c, d and S3d-S3f). This qualitative agreement between mass spectrometric and microscopic results is in the case of \( L \)-His even more unequivocal than it used to be with the remaining \( \alpha \)-amino acids scrutinized in our earlier studies [1–5]. Heavy water evidently obstructs both, formation of the lower (soluble) and higher (insoluble) \( L \)-His-derived peptides.

**Conclusion**

This is the sixth consecutive report from our series focused on investigating an impact of heavy water on oscillatory or fluctuating peptidization patterns with proteinogenic \( \alpha \)-amino acids, and this time the examined species is \( L \)-His. In the previous five cases, we targeted \( L \)-Cys, \( L \)-Met, \( L \)-Pro, \( L \)-Hyp, and \( L \)-Ala. Once again, the experimental evidence collected for \( L \)-His exposes a very strong hampering effect of \( D_2O \) on the fluctuating peptidization scheme of this proteinogenic \( \alpha \)-amino acid.

In all six cases, we observed the oscillatory or fluctuating nature (understood as alternating concentration changes of the considered \( \alpha \)-amino acid monomers), indicating changeable formation and hydrolytic decay of the oligopeptides in the function of time. Moreover, with two endogenous species (\( L \)-Cys and \( L \)-Pro), we

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**Fig. 2** Scanning electron micrographs recorded for the \( L \)-His-derived peptides retrieved from the samples dissolved in MeOH + \( X \), 70:30 (v/v), where \( X \) : the binary mixture of \( H_2O + D_2O \) in the changing volume proportions; a 0% \( D_2O \), \( \times 200 \); b 10% \( D_2O \), \( \times 200 \); c 30% \( D_2O \), \( \times 200 \); d \( L \)-His dissolved in pure 100% \( D_2O \), \( \times 200 \)
observed circadian periodicity of oscillations, with the periods of ca. 24 h and ca. 20 h, respectively. We realize that our results cover the tip of the iceberg only with reference to periodicity (or otherwise) of oscillatory peptidization with proteinogenic α-amino acids, or to their fluctuating peptidization. The reason is that so far, we tested a limited number of six proteinogenic α-amino acids (out of 22 compounds), and the tests were carried out at a single working temperature and using one solvent type only. It cannot be excluded that the investigated compounds demonstrate periodicity (circadian or other) under different working conditions and that periodicity of oscillatory peptidization is inherent of some other, not yet investigated α-amino acids as well.

The HPLC measurements resulting in the time series (Fig. S2a) are the only ones to dispense quantitative data, whereas the MS and SEM data are of a qualitative importance only. The employed working temperature of the HPLC experiment was equal to 22.0 ± 0.5 °C, which with most mammals is considerably below their normal body temperature yet on the other hand, it has been confirmed that quite a number of mammals are able to hibernate for weeks, or even months in the environments much below the freezing point, apparently with the preserved living processes. Hence, a question arises, whether oscillations and fluctuations observed with aid of HPLC for the monomeric L-His (and for five earlier investigated proteinogenic α-amino acids) are of any relevance to the real physiological processes running in the nature. Maybe a partial answer to this question is that we have collected similar HPLC data for several proteinogenic α-amino acids at 30 °C (unpublished results) and in that case, analogical processes of a fluctuating decrease of the monomer concentration at an expense of the oligopeptide formation were observed. Summing up, we are rather convinced that these processes take place within a broad enough range of temperatures, although—similar to the majority of other chemical processes—with the rates of oscillations and fluctuations subdued to the Arrhenius equation.

Tracing periodicity is both time-consuming and quite expensive (considering the costs of equipment, consumables etc.) Nevertheless, an investment in a more detailed scrutiny of spontaneous oscillatory or fluctuating chiral conversion and peptidization of α-amino acids might result in hard to overestimate conclusions relevant, e.g., to molecular biology. Investigations on periodicity of the oscillatory peptidization of proteinogenic α-amino acids target molecular level of this phenomenon and the results might perhaps help understand kinetics of various different metabolic cycles, both in humans and animals. Viewed from a broader perspective, this kind of research might also contribute to a discussion on possible formation of peptides in the prebiotic period of Earth and on origins of biological Life, or contribute to considerations on homochirality of higher living organisms (which otherwise becomes increasingly debatable, due to a substantial improvement of analytical tools).

Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s11144-021-02003-x.

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References

1. Fulczyk A, Łata E, Dolnik M, Talik E, Kowalska T, Sajewicz M (2018) Impact of D$_2$O on peptidization of L-cysteine. Reac Kinet Mech Cat 125:555–565
2. Fulczyk A, Łata E, Talik E, Kowalska T, Sajewicz M (2019) Impact of D$_2$O on peptidization of L-proline. Reac Kinet Mech Cat 128:599–610
3. Fulczyk A, Łata E, Talik E, Dolnik M, Kowalska T, Sajewicz M (2020) Impact of D$_2$O on peptidization of L-alanine. Reac Kinet Mech Cat 130:5–15
4. Fulczyk A, Łata E, Talik E, Kowalska T, Sajewicz M (2019) Impact of D$_2$O on peptidization of L-methionine. Reac Kinet Mech Cat 126:939–949
5. Fulczyk A, Łata E, Talik E, Kowalska T, Sajewicz M (2020) Impact of D$_2$O on peptidization of L-hydroxyproline. Reac Kinet Mech Cat 129:17–28
6. Fulczyk A, Łata E, Talik E, Kowalska T, Sajewicz M (2020) The hampering effect of heavy water (D$_2$O) on oscillatory peptidization of selected proteinogenic α-amino acids. Front Chem 8:541. https://doi.org/10.3389/fchem.2020.00541
7. Sajewicz M, Piętka R, Pieniak A, Kowalska T (2005) Application of thin-layer chromatography (TLC) to investigating oscillatory instability of the selected profen enantiomers. Acta Chromatogr 15:131–149
8. Sajewicz M, Gontarska M, Kronenbach D, Wojtal L, Grygiernczyk G, Kowalska T (2007) Study of the oscillatory in vitro transenantionomerization of the antimers of flurbiprofen and their enantioseparation by thin-layer chromatography (TLC). Acta Chromatogr 18:226–237
9. Sajewicz M, Gontarska M, Kronenbach D, Kowalska T (2008) Investigation of the spontaneous oscillatory in vitro chiral conversion of L-(+)-lactic acid. Acta Chromatogr 20:209–225
10. Sajewicz M, Kronenbach D, Gontarska M, Wróbel M, Pietka R, Kowalska T (2009) TLC in search for structural limitations of spontaneous oscillatory in-vitro chiral conversion. α-hydroxybutyric and mandelic acids. J Planar Chromatogr 22:241–248
11. Sajewicz M, Matlengiewicz M, Leda M, Gontarska M, Kronenbach D, Kowalska T, Epstein IR (2010) Spontaneous oscillatory in vitro chiral conversion of simple carboxylic acids and its possible mechanism. J Phys Org Chem 23:1066–1073
12. Godziek A, Maciejowska A, Talik E, Wrzalik R, Sajewicz M, Kowalska T (2016) On spontaneously pulsating proline-phenylalanine peptide microfibers. Curr Prot Pept Sci 17:106–116
13. Godziek A, Maciejowska A, Talik E, Sajewicz M, Kowalska T (2016) Scanning electron microscopic evidence of spontaneous heteropeptide formation in abiotic solutions of selected α-amino acid pairs. Isr J Chem 56:1057–1066
14. Stich M, Blanco C, Hochberg D (2013) Chiral and chemical oscillations in a simple dimerization model. Phys Chem Chem Phys 15:255–261
15. Sajewicz M, Wzalik R, Gontarska M, Kronenbach D, Leda M, Epstein IR, Kowalska (2009) In vitro chiral conversion, phase separation, and wave propagation in aged profen solutions. J Liq Chromatogr Relat Technol 32:1359–1372
16. Sajewicz M, Gontarska M, Kronenbach D, Leda M, Kowalska T, Epstein IR (2010) Condensation oscillations in the peptidization of phenylglycine. J Syst Chem 1:7. https://doi.org/10.1186/1759-2208-1-7
17. Sajewicz M, Dolnik M, Kowalska T, Epstein IR (2014) Condensation dynamics of L-proline and L-hydroxyproline in solution. RSC Adv 4:7330–7339
18. Maciejowska A, Godziek A, Talik E, Sajewicz M, Kowalska T, Epstein IR (2016) Spontaneous pulsation of peptide microstructures in an abiotic liquid system. J Chromatogr Sci 54:1301–1309
19. Peacock-Lopez E, Bock W (2020) Chiral oscillations and spontaneous mirror symmetry breaking in a simple polymerization model. Symmetry 12(9), 1388. https://doi.org/10.3390/sym12091388
20. Rose WC, Haines WJ, Warner DT, Johnson JE (1951) The amino acid requirements of man. II. The role of threonine and histidine. J Biol Chem 188:49–58
21. Rose WC, Haines WJ, Warner DT (1951) The amino acid requirements of man. III. The role of isoleucine: additional evidence concerning histidine. J Biol Chem 193:605–612
22. Cho ES, Anderson HL, Wixom RL, Hanson KC, Krause GF (1984) Long-term effects of low histidine intake on men. J Nutr 114:369–384
23. Nasset ES, Gatewood VH (1954) Nitrogen balance and hemoglobin of adult rats fed amino acid diets low in L- and D-histidine. J Nutr 53:163–176
24. Clemens RA, Kopple JD, Swendseid ME (1984) Metabolic effects of histidine-deficient diets fed to growing rats by gastric tube. J Nutr 114:2138–2146
25. Kopple JD, Swendseid ME (1975) Evidence that histidine is an essential amino acid in normal and chronically uremic man. J Clin Invest 55:881–891
26. Fukuda S, Kopple JD (1979) Evidence that dog kidney is an endogenous source of histidine. Am J Physiol 237(1):E1–E5; or Am J Physiol: Endocrinol Metab Gastrointest Physiol 6(1):E1–E5
27. FAO/WHO/UNU (1985) Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. WHO Technical Report Series 724. WHO, Geneva, Switzerland
28. Kriengsinyos W, Rafii M, Wykes LJ, Ball RO, Pencharz (2002) Long-term effects of histidine depletion on whole-body protein metabolism in healthy adults. J Nutr 132:3340–3348
29. Hou Y, Yin Y, Wu G (2015) Dietary essentiality of “nutritionally non-essential amino acids” for animals and humans. Exp Biol Med (Maywood) 240:997–1007

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