Supporting Information

Electrocatalytic Monitoring of Peptidic Proton-Wires

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Section 1: Electrochemistry

Peptides (purity > 98 %) were custom synthesised by Clonestar Peptide Services Ltd (CZ). Other chemicals and water were ACS reagents purchased from Sigma-Aldrich. An Autolab analyser (Eco Chemie) in connection with VA-stand 663 (Metrohm) was used for chronopotentiometric measurements. The three-electrode system consisted of HMDE as a working electrode (area of 0.4 mm^2), Ag|AgCl|3 M KCl as a reference electrode, and Pt wire as a counter electrode. All chronopotentiometric measurements were performed at laboratory temperature open to air. Peptides were accumulated on the electrode from unstirred solutions for a 60 s accumulation time (t_A) at an accumulation potential (E_A) of +0.1 V. After accumulation, chronopotentiograms were recorded in the same solution from an initial potential ($E_i = E_A$). Microprocessor-based Bench pH Meter 210 and pH-electrode BioTrode from Hanna Instruments (Italy) and from Hamilton (Switzerland) were used, respectively.

S1.1. Effect of HA2 and H6 concentration on peak H characteristics.

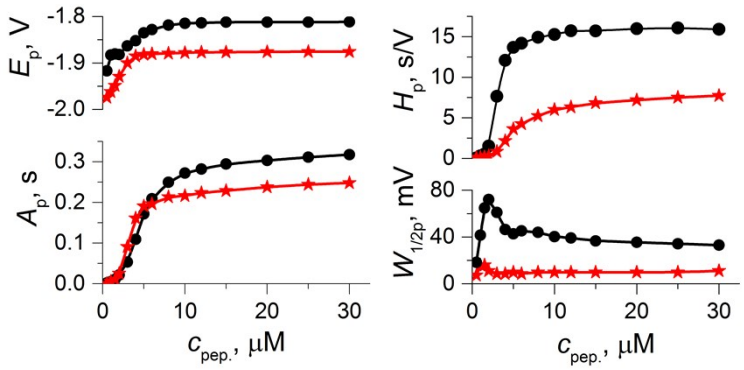

Figure S1. Dependences of HA2 (red) and H6 (black) peak H potential (E_p), area (A_p), height (H_p), and half width ($W_{1/2p}$) on peptide concentration ($c_{pep.}$) at I_{str} of -25 µA.

S1.2. Stripping current (I_{str}) effects

In chronopotentiometry, the reciprocal value of the H_p indicates the rate ($\nu_p = 1/H_p$) of the electrode polarisation at E_p, *i.e.* at the potential of the most effective proton transport in the case of CPS peak H. While the A_p, corresponding with a transition time ($\tau = A_p$), is proportional to the amount of the electrocatalytically generated molecular hydrogen. It can be used to calculate the $I_{str}\tau^{1/2}$-value that is for a well-behaved system independent of I_{str} according to the Sand equation describing constant-current electrolysis [Bard, A.J. and L.R. Faulkner, Controlled-current techniques, in Electrochemical Methods: Fundamentals and Applications 2001, Wiley p. 310].

In Figure S2, showing I_{str}-dependences of ν_p and $I_{str}\tau^{1/2}$-values for all four investigated peptides at a 50 mM $c_{buf.}$, the used I_{str} range is divided into three sections according to the breaks indicated on the ν_p vs. I_{str}-plot of HA2 that correspond well with those on its $I_{str}\tau^{1/2}$ vs. I_{str}-plot. Breaks at I_{str} of –35 and –95 μA are cutting off the transition region in which both unfolded and folded molecules of HA2 participated in the CHER. However, using I_{str}'s more negative than –95 µA, where only folded molecules of HA2 mediated transport of protons from the solution proton donor BH onto the electrode surface, peaks H of HA3 and HA4 were only poorly developed and merged with a discharge of the supporting electrolyte.

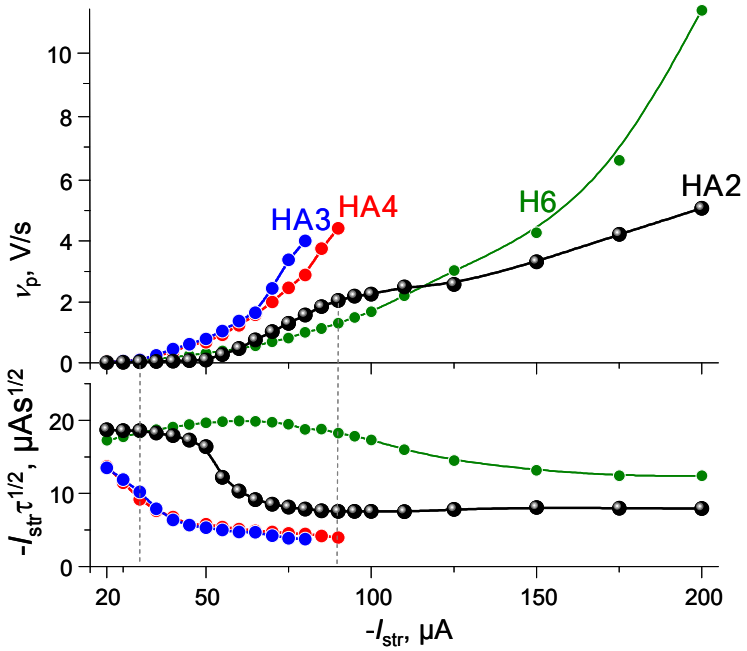

Figure S2. Dependences of ν_p and $I_{str}\tau^{1/2}$-values of HA2, HA3, HA4 and H6 on I_{str} at 50 mM concentration of Na-phosphate buffer solution of pH 6.

On the other hand, in the case of a random coil H6 molecule, producing well-developed peaks H over the entire used I_{str}-range, no significant deviation from a monotonous change of the ν_p and $I_{str}\tau^{1/2}$-value was detected. Observed increase of the ν_p or decrease of the $I_{str}\tau^{1/2}$-value of H6 with increasing cathodic I_{str} intensity is consistent with a catalytic character of the underlying electrode process involving the slow irreversible reduction of a proton from the imidazolium group connected to the reprotonation (regeneration) of the resulting imidazole group by a slightly acidic proton donor BH from the solution. While the initial increase of the $I_{str}\tau^{1/2}$-value of H6 at low cathodic I_{str} intensities, from –20 to –60 μA might be attributed to its dynamic interaction with the electrode surface due to slow polarising rates of the electrode allowing reorientation of its molecules in adsorbed state, as it was observed earlier in the case of some peptides and proteins [Electroanalysis, 2009, 21(3-5) 662-665].

Presented results shows that using CPS peak H it was possible to distinguish between the folded and unfolded forms of helical peptides HA2, HA3, and HA4 adsorbed at the electrode surface.

Section 2: Theoretical Investigations

Initial structures of the HA2 and H6 and both in 3_{10}- and α-helical conformations were generated by program Hyperchem. In each helical structure the N-terminus was capped with an acetyl group, whereas the C-terminus was capped with an amino group. Histidines were taken as neutral species with hydrogen situated at the ε-nitrogen.

All calculations were performed using the TURBOMOLE 6.3 program. The *ab initio* technique was employed for all atoms of the systems studied, contrary to the work of Lopez *et al.* [J. Phys. Chem. A. 116 (2012) 1283]. The BLYP functional and the SVP basis set were used systematically through the study. The optimization procedure was performed in vacuum and also in the implicit solvent (COSMO model with default parameters) to cover the role of water at least qualitatively. To speed up the calculations the Resolution of Identity approximation was used. A character of optimized structures and transition states found was verified by performance of a harmonic vibrational analysis.

The proton transfer ability of the systems was studied in the same way, as it was utilized by Lopez *et al.* [J. Phys. Chem. A. 116 (2012) 1283]. In the optimized neutral structure of HA2 the histidines were protonated at δ nitrogen position and the structure was reoptimized (Fig. 5 in the main text and Fig. S3).

Results – Theoretical Investigations

Geometrical properties

The H6 backbone geometry remains unchanged from ideal parameters after optimization in vacuum and also in implicit water for both types of conformations. For further details see Table S1a. Bigger changes during the optimization procedure were observed for the geometry of His side chains. A tendency of these side residues to create a compact H-bond network between themselves is a reason for it.

For HA2 among several initial conformations of side chains of histidines, the most stable structure after optimization corresponds to the geometry, where all histidines are involved in the continuous H-bond network. For this peptide the tendency of alanine residues to maintain the α-helical conformation is visible and thus the deviations from the ideal 3_{10}-helix are more distinctive for the structure (see Table S1b). Moreover, the optimized 3_{10}-helical structure rather resembles an intermediate between alpha and 3_{10}- helix according to the Ramachandran's plot and the localization of H-bond network in the backbone. A rather significant difference in *phi* and *psi* torsion angles of alanine and histidine residues was observed (values for Ala are more "alpha-helical" than the His ones). Water itself has only minor effect on the geometry of the backbone.

The protonated structures do not differ considerably from the unprotonated species especially in the water environment. The structure of the backbone remains practically unchanged for the HA2 (differences of *phi* and *psi* angles of protonated and unprotonated structure do not exceed few of degrees). Larger discrepancies were observed for the 3_{10}-helical conformation than for the α-helix, in agreement with findings of Lopez *et al.* [J. Phys. Chem. A. 116 (2012) 1283]. The reason for it is a closer distance between histidines in 3_{10}-helical form in comparison with α-helical a thus the bigger repulsion between these residues. It leads to the greater variation of the geometry of 3_{10}- conformation during the optimization procedure.

We observed significantly more changes in the conformation of proteins during the optimization in vacuum where the electrostatic forces are stronger. Due to the presence of only few H-bonds stabilizing the secondary structure in H6 these peptides are very flexible and the repulsion of positively charged histidines leads to the extended structure. The majority of H-bonds in initial structure is broken during optimization both in 3_{10}- helical conformation than for the α-helix

A visible rearrangement of the 3-_{10} helical structure of 21-mer polypeptide accompanied also by a kink of the structure was found after minimization.

Table S1a. H6 peptide geometry of ideal and optimized helical structures in vacuum (VAC) and implicit water (WAT). Averaged *Phi and Psi* backbone torsion angles with RMSD are in degrees and their comparison with ideal geometries of helices. Averaged RMSDs in Å between protonated structures and non-protonated one.

	3_{-10} *ideal*	3_{-10} VAC	3_{-10} WAT	α-helix *ideal*	α-helix VAC	α-helix WAT
Phi	*-49.0*	-52.3±5.9	-52.9±2.8	*-57.8*	-57.7±6.3	-60.3±2.9
Psi	-26.0	-28.0±7.2	-29.7±4.7	*-47.0*	-42.8±9.8	-46.1±4.9
Avg. RMSD		0.34	0.07		0.75	0.40

Table S1b. HA2 peptide conformation properties of helical structures in vacuum and implicit water medium. Averaged *Phi and Psi* backbone torsion angles are depicted separately for alanine and histidine residues. Averaged RMSDs in Å between protonated structures and non-protonated one.

	3_{-10} *ideal*	3_{-10} Ala VAC	3_{-10} His VAC	3_{-10} Ala WAT	3_{-10} His WAT	α-helix *ideal*	α-helix Ala VAC	α-helix His VAC	α-helix Ala WAT	α-helix His WAT
Phi	*-49.0*	-57.8	-66.2	-58.7	-68.4	*-57.8*	-58.4	-61.9	-59.2	-60.8
Psi	*-26.0*	-32.8	-22.7	-39.7	-27.3	*-47.0*	-40.3	-33.3	-40.5	-35.4
Avg. RMSD		1.73		1.98			1.09		0.67	

Proton affinities

The proton affinity was calculated by computing the energy difference between the optimized unprotonated molecule and the average value of energies of optimized protonated molecule. The results are summarized in the Table 1 (see the main text).

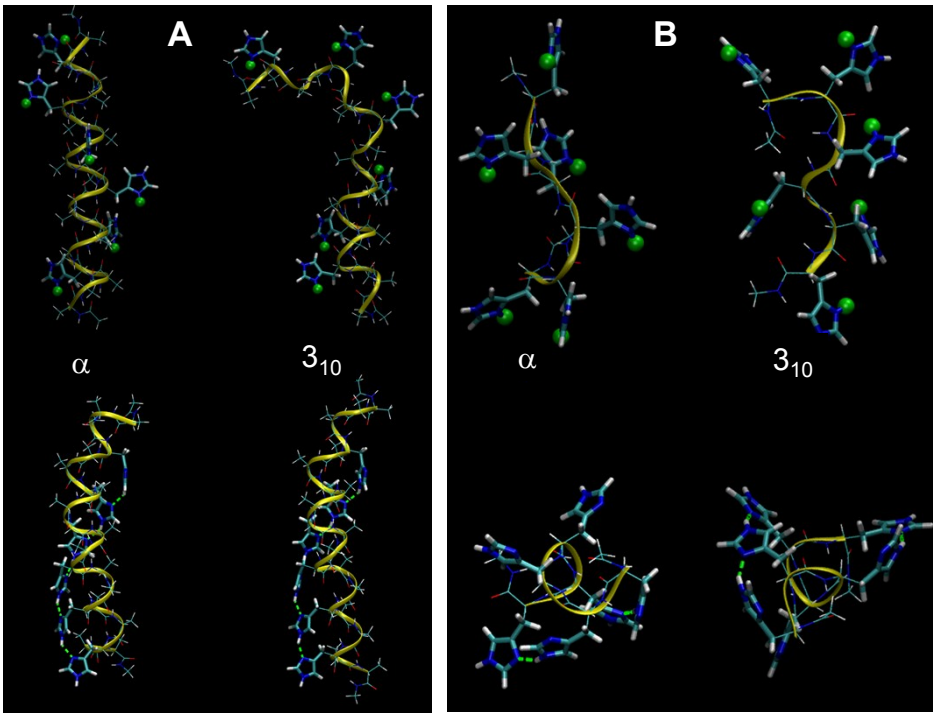

Figure S3. Optimized α- and 3_{10}-structures of HA2 (A) and H6 (B) in protonated (top) and unprotonated (below) state in vacuum. Protons bind to δ nitrogens of His residues are highlighted as a green spheres.

Section 3: Circular dichroism

For measurement of circular dichroism (CD) spectra, H6 and HA2 peptides were diluted to the final concentration of 10 μM in 50 mM Na-phosphate buffer solution. Signal of pure buffer was recorded as a baseline. The spectra were acquired on the J-815 CD spectrometer (Jasco) at 23 °C, in the 190–260 nm interval. Cuvette of 0.1 cm diameter was used. CD signals were expressed as the difference in the molar absorption Δε of the left- and right-handed circularly polarised light. Spectra were acquired at a rate of 50 nm/min and averaged from three measurements.