Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

# Hydrogen-deuterium exchange in imidazole as a tool for studying histidine phosphorylation

Małgorzata Cebo, Martyna Kielmas, Justyna Adamczyk, Marek Cebrat, Zbigniew Szewczuk, Piotr Stefanowicz*

**Table S1**. The comparison of calculated and experimental masses for $[MH]^+$ ions of model peptides and their monodeuterated analogues

| Peptide name | Peptide sequence | Molecular formula | Native peptide Found mass [Da] | Native peptide Calculated mass [Da] | Deuterated peptide Calculated mass [Da] | Deuterated peptide Found mass[Da] |
|---|---|---|---|---|---|---|
| **1RH0** | H-**Ala-Ala-Arg-His-Ala-Phe**-OH | $C_{30}H_{45}N_{11}O_7$ | 672.3764 | 672.3582 | 673.3639 | 673.3893 |
| **2DH0** | H-**Ala-Ala-Asp-His-Ala-Phe**-OH | $C_{28}H_{38}N_8O_9$ | 631.2815 | 631.2835 | 632.2897 | 632.3107 |
| **3RH1** | H-**Ala-Arg-Ala-His-Ala-Phe**-OH | $C_{30}H_{45}N_{11}O_7$ | 672.3822 | 672.3582 | 673.3639 | 673.3882 |
| **4DH1** | H-**Ala-Asp-Ala-His-Ala-Phe**-OH | $C_{28}H_{38}N_8O_9$ | 631.2805 | 631.2835 | 632.2897 | 632.3134 |
| **5RH2** | H-**Arg-Ala-Ala-His-Ala-Phe**-OH | $C_{30}H_{45}N_{11}O_7$ | 672.3822 | 672.3582 | 673.3639 | 673.3891 |
| **6DH2** | H-**Asp-Ala-Ala-His-Ala-Phe**-OH | $C_{28}H_{38}N_8O_9$ | 631.3058 | 631.2835 | 632.2897 | 632.3114 |
| **70H0** | H-**Ala-Ala-Ala-His-Ala-Phe**-OH | $C_{27}H_{38}N_8O_7$ | 587.3133 | 587.2936 | 588.2999 | 588.3195 |

**Table S2**. The selected fragment ions for model peptides

| Peptide name | Peptide sequence | Ion fragments | Found mass [Da] | Calculated mass [Da] |
|---|---|---|---|---|
| **1RH0** | H-**Ala-Ala-Arg-His-Ala-Phe**-OH | b4 | 436.2427 | 436.2421 |
| | | y3 | 374.1855 | 374.1829 |
| **2DH0** | H-**Ala-Ala-Asp-His-Ala-Phe**-OH | b5 | 466.2195 | 466.2051 |
| | | y4 | 489.2253 | 489.2098 |
| **3RH1** | H-**Ala-Arg-Ala-His-Ala-Phe**-OH | a5 | 479.2995 | 479.2843 |
| | | b4 | 436.3552 | 436.2421 |
| **4DH1** | H-**Ala-Asp-Ala-His-Ala-Phe**-OH | b6 | 613.2953 | 613.2735 |
| | | y4 | 445.2333 | 445.2200 |
| **5RH2** | H-**Arg-Ala-Ala-His-Ala-Phe**-OH | a5 | 479.2996 | 479.2843 |
| | | b4 | 436.2552 | 436.2421 |
| **6DH2** | H-**Asp-Ala-Ala-His-Ala-Phe**-OH | b4 | 395.1675 | 395.1680 |
| | | y3 | 374.1846 | 374.1829 |
| **70H0** | H-**Ala-Ala-Ala-His-Ala-Phe**-OH | b5 | 422.2274 | 422.2152 |
| | | y4 | 445.2334 | 445.2200 |

**Table S3**. The comparison of calculated and experimental masses for $[MH]^+$ ions of monodeuterated analogues of phosphorylated peptides

| Peptide name | Peptide sequence | Peptide with pHis Calculated mass | Deuterated peptide with pHis Calculated mass | Deuterated peptide with pHis Found mass |
|---|---|---|---|---|
| **1RH0** | H-**Ala-Ala-Arg-His-Ala-Phe**-OH | 752.3239 | 753.3302 | 753.3595 |
| **2DH0** | H-**Ala-Ala-Asp-His-Ala-Phe**-OH | 711.2498 | 712.2561 | 712.2833 |
| **3RH1** | H-**Ala-Arg-Ala-His-Ala-Phe**-OH | 752.3239 | 753.3302 | 753.3604 |
| **4DH1** | H-**Ala-Asp-Ala-His-Ala-Phe**-OH | 711.2498 | 712.2561 | 712.2844 |
| **5RH2** | H-**Arg-Ala-Ala-His-Ala-Phe**-OH | 752.3239 | 753.3302 | 753.3606 |
| **6DH2** | H-**Asp-Ala-Ala-His-Ala-Phe**-OH | 711.2498 | 712.2561 | 712.2826 |
| **70H0** | H-**Ala-Ala-Ala-His-Ala-Phe**-OH | 667.2600 | 668.2662 | 668.2905 |

**Table S4.** CID fragmentation pattern for peptide **DRVYJHPF**

| Fragment | Molecular formula | Calculated m/z | Experimental m/z |
|---|---|---|---|
| $y_2^+$ | $C_{14}H_{19}N_2O_3$ | 263.1239 | 263.1451 |
| $[b_6]^{2+}$ | $C_{36}H_{55}N_{11}O_9$ | 393.2131 | 393.2232 |
| $b_4^+$ | $C_{24}H_{36}H_7O_7$ | 534.2676 | 534.2853 |
| $a_5^+$ | $C_{29}H_{47}N_8O_7$ | 619.3568 | 619.3791 |
| $b_5^+$ | $C_{30}H_{47}N_8O_8$ | 647.3517 | 647.3753 |
| $[y_6\text{-}H_2O]^+$ | $C_{40}H_{53}N_8O_7$ | 757.4037 | 757.4519 |
| $[b_6\text{-}NH_3]^+$ | $C_{36}H_{50}DN_{10}O_9$ | 768.4005 | 768.4219 |
| $b_6^+$ | $C_{36}H_{53}DN_{11}O_9$ | 785.4169 | 785.4489 |

**Table S5. CID fragmentation pattern for peptide NHFWKTHT**

| Fragment | Molecular formula | Calculated m/z | Experimental m/z |
|---|---|---|---|
| $[b_2\text{-}NH_3]^+$ | $C_{10}H_{11}N_4O_3$ | 235.0863 | 235.0822 |
| $b_2^+$ | $C_{10}H_{14}N_5O_3$ | 252.1097 | 252.1088 |
| $[y_3\text{-}NH_3]^+$ | $C_{14}H_{19}DN_5O_5$ | 341.1715 | 341.1840 |
| $y_3^+$ | $C_{14}H_{22}DN_6O_5$ | 358.1949 | 358.1884 |
| $b_3^+$ | $C_{19}H_{23}N_6O_4$ | 399.1781 | 399.1783 |
| $[b_4\text{-}NH_3]^+$ | $C_{30}H_{30}N_7O_5$ | 568.2340 | 568.2369 |
| $b_4^+$ | $C_{30}H_{33}N_8O_5$ | 585.2574 | 585.2603 |
| $[z_5\text{-}H_2O]^+$ | $C_{31}H_{41}DN_9O_6$ | 637.3322 | 637.3296 |
| $[y_5\text{-}H_2O]^+$ | $C_{31}H_{42}DN_{10}O_6$ | 654.3587 | 654.3570 |
| $y_5^+$ | $C_{31}H_{44}DN_{10}O_7$ | 672.3692 | 672.3675 |
| $b_5^+$ | $C_{36}H_{45}N_{10}O_6$ | 713.3524 | 713.3581 |
| $[z_6\text{-}H_2O]^+$ | $C_{40}H_{50}DN_{10}O_7$ | 784.4006 | 784.4070 |
| $[y_6\text{-}H_2O]^+$ | $C_{40}H_{51}DN_{11}O_7$ | 801.4271 | 801.4344 |
| $y_6^+$ | $C_{40}H_{53}DN_{11}O_8$ | 819.4376 | 819.4451 |

**Table S6.** CID fragmentation pattern for peptide **HYIQNHPLG**

| Fragment | Molecular formula | Calculated m/z | Experimental m/z |
|---|---|---|---|
| $y_3^+$ | $C_{13}H_{25}N_4O_3$ | 285.1770 | 295.1994 |
| $b_2^+$ | $C_{15}H_{16}DN_4O_3$ | 302.1364 | 302.1434 |
| $[b_6]^{2+}$ | $C_{36}H_{49}DN_{12}O_9$ | 398.1982 | 398.1910 |
| $b_3^+$ | $C_{21}H_{27}DN_5O_4$ | 415.2204 | 415.2261 |
| $[a_8]^{2+}$ | $C_{46}H_{67}DN_{14}O_{10}$ | 489.2692 | 489.2126 |
| $[b_8]^{2+}$ | $C_{47}H_{67}DN_{14}O_{11}$ | 503.2666 | 503.2787 |
| $[b_5\text{-}H_2O]^+$ | $C_{30}H_{39}DN_9O_8$ | 639.3114 | 639.3002 |
| $b_5^+$ | $C_{30}H_{41}DN_9O_8$ | 657.3219 | 657.3404 |
| $[b_6\text{-}CO]^+$ | $C_{35}H_{48}DN_{12}O_8$ | 766.3859 | 766.3432 |
| $y_7^+$ | $C_{34}H_{55}N_{12}O_9$ | 777.4215 | 777.4690 |
| $b_6^+$ | $C_{36}H_{48}DN_{12}O_9$ | 794.4140 | 794.4104 |
| $z_8^+$ | $C_{43}H_{62}DN_{12}O_{11}$ | 923.5039 | 923.5138 |
| $y_8^+$ | $C_{43}H_{64}DN_{13}O_{11}$ | 940.5148 | 940.5438 |

## IMAGES

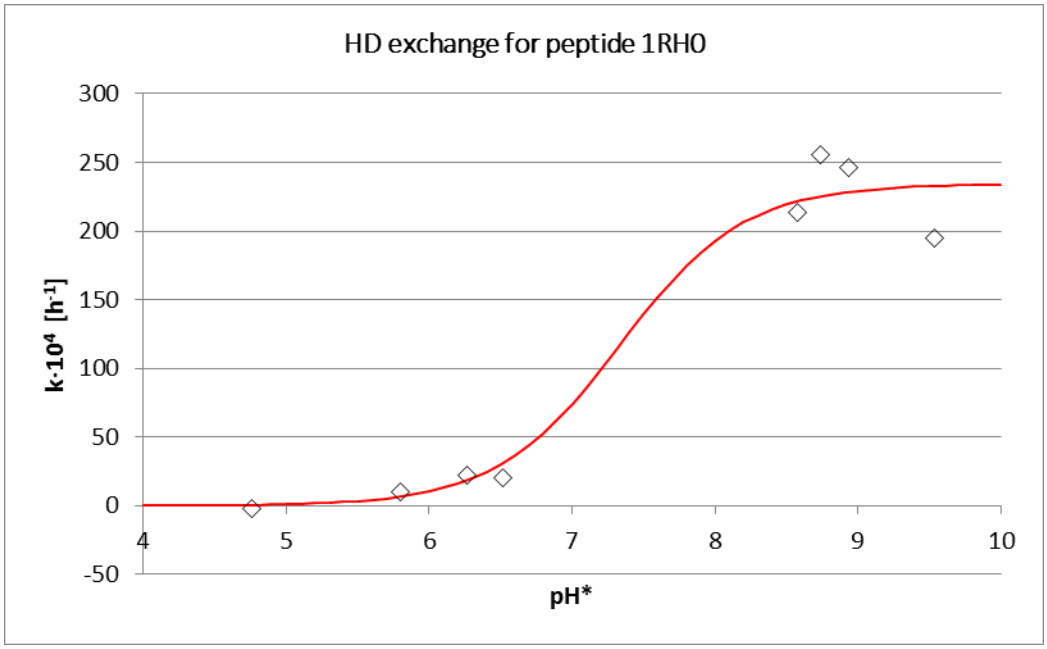


**Fig. S1. The dependence of HDX rate constant on pH for peptide 1RH0**

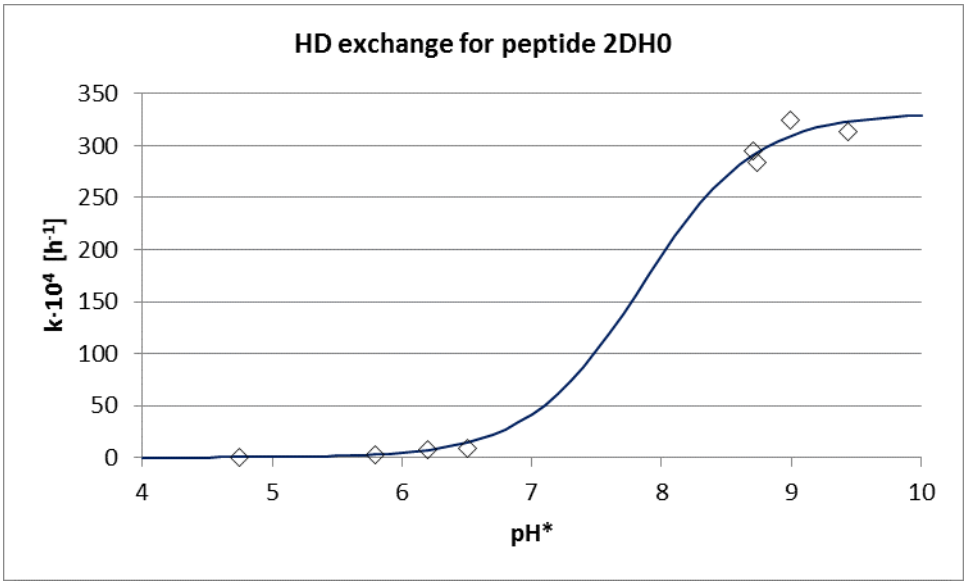


**Fig. S2. The dependence of HDX rate constant on pH for peptide 2DH0**

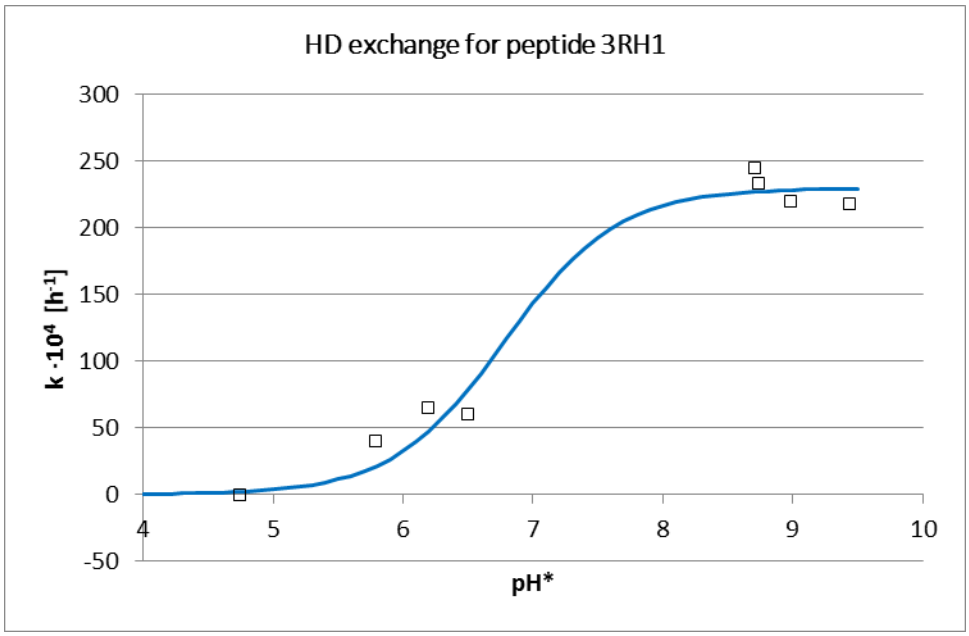


**Fig. S3. The dependence of HDX rate constant on pH for peptide 3RH1**

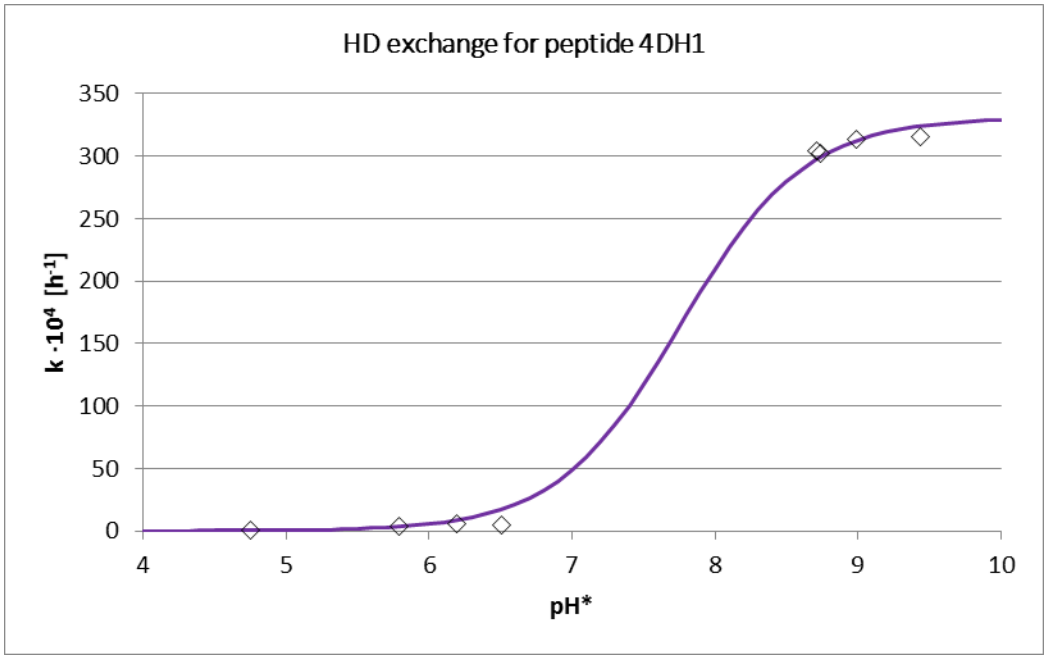


**Fig. S4. The dependence of HDX rate constant on pH for peptide 4DH1**

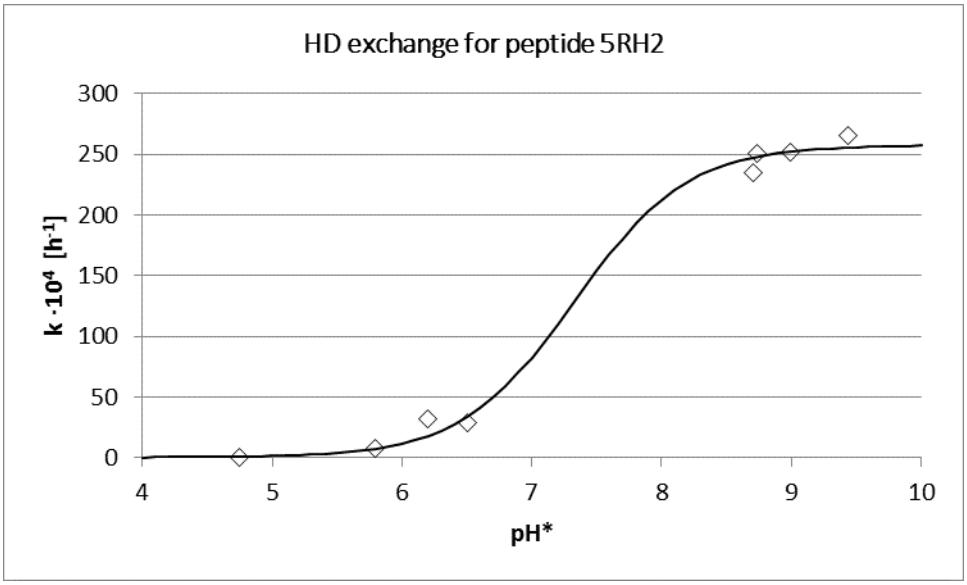


**Fig. S5. The dependence of HDX rate constant on pH for peptide 5RH2**

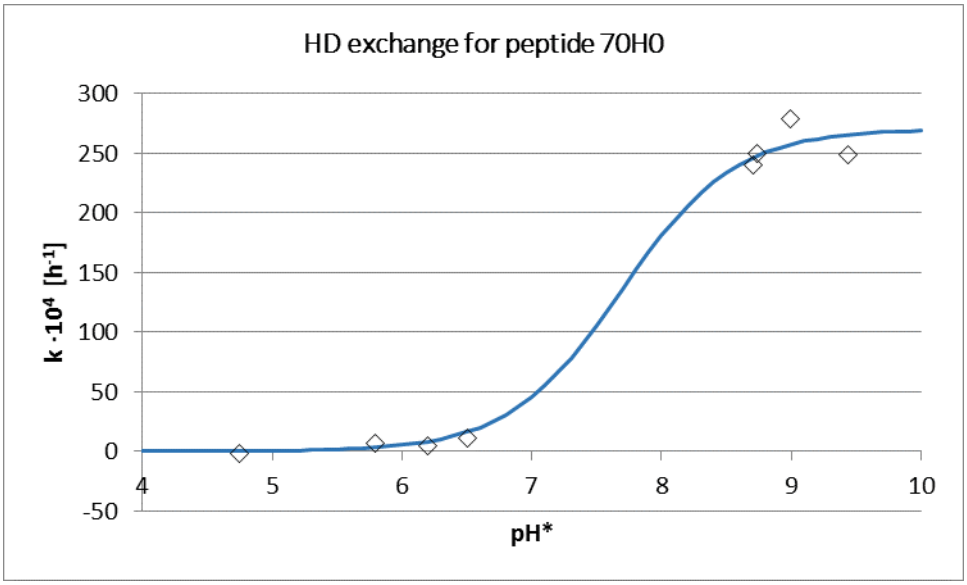


**Fig. S6. The dependence of HDX rate constant on pH for peptide 70H0**

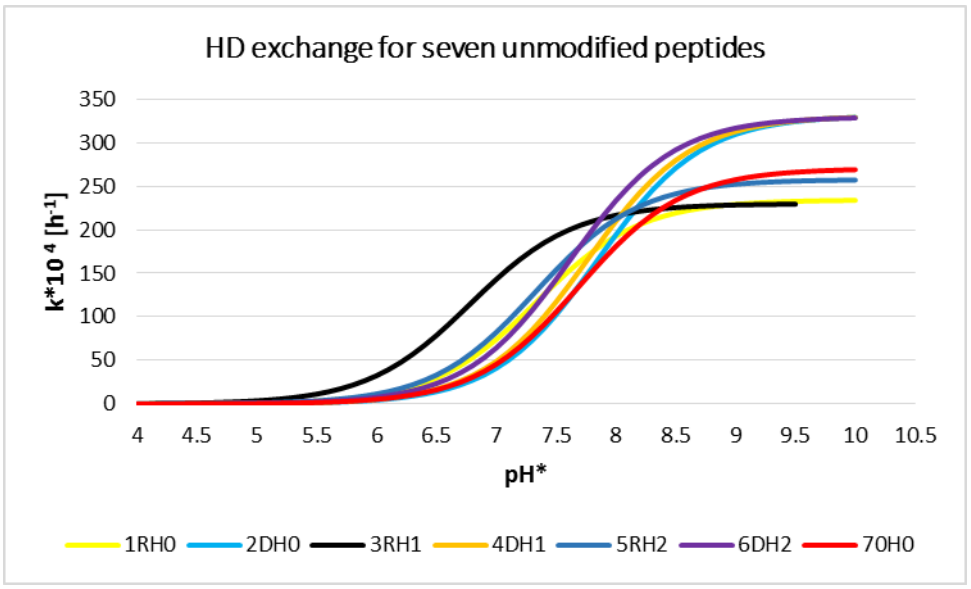


**Fig. S7. The dependence of HDX rate constant on pH for all seven unmodified peptide**