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Histone Tail Sequences Balance Their Role in Genetic Regulation and the Need to Protect DNA Against Destruction in Nucleosome Core Particles Containing Abasic Sites

Dr. Kun Yang^[a] [Professor] and Marc M. Greenberg^[a]

^[a]Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218

Abstract

Abasic sites (AP) are produced 10,000 times in a single cell per day. Strand cleavage at AP is accelerated ~100-fold within a nucleosome core particle (NCP) compared to free DNA. The lysine (Lys) rich N-terminal tails of histone proteins catalyze single strand break formation via a mechanism utilized by base excision repair enzymes, despite the general dearth of Glu, Asp, and His amino acids that are typically responsible for deprotonation of Schiff base intermediates. Incorporating Glu, Asp, or His proximal to Lys residues in histone N-terminal tails increases AP reactivity as much as ~6-fold. The rate acceleration is due to more facile DNA cleavage of Schiff base intermediates. These observations raise the possibility that histone proteins may have evolved to minimize the presence of His, Glu, and Asp in their Lys rich N-terminal tails to guard against enhancing the toxic effects of DNA damage.

Graphical Abstract

Histone proteins catalyze DNA strand scission at abasic sites within nucleosome core particles. Lysine residues that are responsible for this detrimental chemistry are necessary for other functions within chromatin. However, histone proteins may have evolved to minimize the levels of other amino acids (His, Glu and Asp) that further accelerate strand scission.

Correspondence to: Marc M. Greenberg.

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Nucleosome core particles (NCPs) are the monomeric component of chromatin, the structure in which nuclear DNA is compacted within nuclei. NCPs consist of ~145 base pairs (bps) of DNA wrapped ~1.6 times around an octameric core of highly positively charged histone proteins (Figure 1A). The octameric core is comprised of a tetramer of histones H3 and H4 bound by two dimers of histones H2A and H2B.^[1] Posttranslational modifications of the histone proteins are critical for regulating cellular processes, including chromatin remodeling and gene expression.^[2] Histone proteins within NCPs also react with and influence the reactivity of damaged DNA.^[3] Abasic sites (AP), of which 10,000 are produced per day per cell as a result of normal respiration, are the most common DNA lesion.^[4] DNA undergoes strand scission at AP and oxidized abasic sites via elimination as much as 1500-times faster within nucleosome core particles than the identical sequence of DNA does in aqueous buffer under physiological conditions.^[5] Catalyzing strand scission of its own genomic DNA is deleterious to a cell. Is it possible that the N-terminal histone tail sequences evolved so as to minimize their ability to cleave DNA at the most ubiquitous of lesions, an AP site but yet retain their ability to control biochemical processes that require chemically reactive amino acids?

The unstructured, lysine (Lys, K) rich histone N-amino terminal tails are responsible for the bulk of accelerated AP cleavage in NCPs (Scheme 1).^[5a, 6] The minimal mechanism entails reversible Schiff base (DPC_{un}) formation and rate limiting β -elimination (k_2) to form DPC_{cl} enroute to single strand breaks (SSB). Several enzymes involved in base excision repair (BER), such as DNA polymerase β catalyze this reaction even more efficiently.^[7] Such enzymes activate the abasic site for elimination via Schiff base formation (Scheme 1). Schiff base deprotonation is often attributed to a carboxylate containing glutamic (Glu, E) or aspartic acid (Asp, D) residue. Other families of lyase enzymes utilize histidine (His, H) as a Bronsted base.^[8] The histone amino terminal tail regions are rich in Lys residues that form Schiff bases with AP, but Glu and Asp residues, as well as His are underrepresented compared to their overall presence in mammalian proteomes (Table S1). We considered whether modified histone proteins containing Glu, Asp, and His residues in the N-terminal tails exhibit higher lyase activity, and if so whether this has any bearing on the amino acid content of naturally occurring histones?

Two regions of the NCP (Figure 1A–C) were examined to probe the effects of modifying tail sequences on AP lyase activity. AP_{123} is proximal to the 31-residue long histone H2B tail (Figure 1B, D), which contains a concentrated region of positively charged amino acids ($\text{Lys}_{24} - \text{Lys}_{31}$), known as the histone H2B repression (HBR) domain.^[9] The three arginine (Arg, R) residues (R26, R27 and R30) and four lysine residues (K24, K25, K28 and K31) within this domain are proximal to AP_{123} (Figure 1B). AP_{89} is positioned within a region in which the nucleosomal DNA is kinked and is a hot spot for molecules that bind to (and in some instances damage) the biopolymer.^[10] AP_{89} is proximal to the H4 tail and its reactivity in this NCP region (Figure 1C, D) was investigated to establish the general lyase mechanism (Scheme 1).^[5a, 6, 11] In addition, the unstructured histone H4 N-terminal tail catalyzes strand scission at AP sites distributed over several base pairs ($>25 \text{ \AA}$), indicating the breadth of this process.^[11] NCPs containing AP_{89} or AP_{123} were prepared using the general method previously described.^[5a, 11] Briefly, the 145 nt long oligonucleotides, whose sequences were based upon the Widom 601 strong positioning DNA, were prepared by ligating chemically

synthesized oligonucleotides, one of which contained a photolabile precursor (**1**, Scheme 2) to AP, and purified by denaturing PAGE.^[12] *Xenopus laevis* histone proteins were expressed in *E. coli* and purified by ion exchange chromatography.^[13] The plasmids expressing the histone variants were generated by molecular cloning or site-directed mutagenesis. The histone mutants were expressed and purified in the same manner as the wild type proteins. The NCPs were reconstituted via the salt-dilution method, and characterized by gel electrophoresis and DNase I footprinting.^[13] AP sites were generated immediately prior to the experiment via brief (15 min) photolysis. Rate constants for AP disappearance (k_{Dis} , Table 1, 2) and strand scission (k_{Cl} , Table S2, S3) were determined by measuring the amounts of intact AP, DPC_{un} , DPC_{cl} , and SSB as a function of time via SDS PAGE.

The rate constant of AP_{123} disappearance in NCPs containing wild type histones was $(11.6 \pm 1.4) \times 10^{-6} \text{ s}^{-1}$ (Table 1). This is one-half as fast as the most reactive position reported, and approximately 4-times faster than the least reactive site in a NCP.^[6a, 11] Deleting the first 23 amino acids (1–23 Del.) of the N-terminal H2B protein, leaving the HBR domain intact modestly reduced the rate constant for AP_{123} disappearance by 33%, suggesting that the residues, presumably the four Lys residues within the HBR domain (Figure 1D) account for the majority of the activity. Indeed, substituting alanine for the 4 Lys residues in the remaining HBR domain (1–23 Del, K24,25,28,31A) significantly reduced AP_{123} reactivity by 76%. This was expected based upon the importance of the lysine residues for activating the abasic site towards strand scission via reversible Schiff base formation. The modest (<30%) increase in reactivity when the arginine (R) residues within the HBR domain are mutated to alanine (1–23 Del, R26,27,30A) indicates that Arg is not involved in Schiff base formation and/or β -elimination. Importantly, replacing any one of the Arg residues with Glu (1–23 Del, R26E; 1–23 Del, R27E; 1–23 Del, R30E) resulted in a >2.5-fold increase in AP_{123} reactivity. In addition, k_{Dis} for the NCP containing 1–23 Del, R26,27,30E in which all 3 HBR domain arginines are replaced by glutamic acid was comparable to the summation of the corresponding rate constants within NCPs in which a single Glu was incorporated. This suggests that the glutamic acids act independent of one another and one can infer that the modest decrease in AP_{123} reactivity upon deleting residues 1–23 (Table 1) is due to the loss of Asp₂₂, which is proximal to the Lys rich HBR domain. Interestingly, the rate constants for AP_{123} disappearance were even greater when His was substituted for Arg. Substituting Arg₃₀ with His increases k_{Dis} almost 4-fold, and H2B containing histidine at position 27 in place of arginine results in an almost 6-fold increase in AP_{123} reactivity. Overall, incorporating a Glu or His residue within the HBR domain of the H2B N-terminal tail significantly increases AP_{123} reactivity in NCPs.

Increased β -elimination (k_2 , Scheme 1) was assumed to be responsible for the accelerated reactivity at AP_{123} upon introduction of a Glu or His residue. The corresponding rate constants for AP_{123} strand scission (k_{Cl} , Table S2) are within 10% of k_{Dis} , regardless of the protein sequences. This indicates that significant increases in k_1 alone are not the source of the greater overall AP_{123} reactivity, as this would result in a larger differential between k_{Dis} and k_{Cl} due to a build-up of DPC_{un} . More direct support for the effects of Glu and His on k_2 were gleaned from experiments carried out in the presence of (10 mM) NaBH₃CN (Table 1). The reducing agent selectively traps DPC_{cl} and DPC_{un} . The latter reaction affects k_{Dis} by

competing with DNA-protein crosslink hydrolysis (k_1 , Scheme 1). Although k_{Dis} is greater for all NCPs in the presence of NaBH_3CN , the increase is smaller in constructs containing His or Glu in the H2B tail HBR domain (positions 24–31), as reflected by the relative rates (Table 1). Furthermore, in each instance the ratio of $\text{DPC}_{\text{un}}:\text{DPC}_{\text{cl}}$ in the presence of NaBH_3CN reaches a steady-state value in NCPs containing AP_{123} (Figure 2A, Figure S7). This ratio is considerably smaller when Glu or His is present in the H2B tail compared to the corresponding protein in which arginine (1–23 Del) or alanine (1–23 Del, R26,27,30A) is present. Thus, both observations indicate that increased AP_{123} reactivity in NCPs when Glu or His is introduced within the N-terminal H2B tail is due to enhanced β -elimination (k_2).

The generality of these effects was probed by examining the reactivity of AP_{89} in NCPs (Table 2), which is proximal to the histone H4 tail. AP_{89} reactivity was examined in NCPs that were comprised of wild type histone H4 in which histidine is present at position 18 of the N-terminal tail, or mutant proteins containing either alanine (H18A), glutamic acid (H18D) or aspartic acid (H18E) at position 18. AP_{89} reactivity in wild type NCPs (His_{18}) and those containing H18A was previously reported, and the data here are within experimental error of those reports.^[6b] Interestingly, His had a similar effect on AP_{89} reactivity as it did on that of AP_{123} . AP_{89} reacted more than 3-times faster when His_{18} (Wild type) was present compared to Ala_{18} . In addition, the relative rate in the presence and absence of NaBH_3CN was the same at the two AP sites, indicating that His_{18} had a similar effect on AP_{89} elimination as did His_{27} and His_{30} in histone H2B on AP_{123} reactivity. AP_{89} reactivity in NCPs containing glutamic (H4-H18E) or aspartic acid (H4-H18D) was intermediate relative to histidine or alanine but within experimental error of each other. In addition, NaBH_3CN trapping experiments (Figure 2B) indicate that the differences in k_{Dis} as a function of histone H4 sequence at position 18 are due to changes in the rate constant for β -elimination (k_2 , Scheme 1). Overall, these data are consistent with the dependence of AP_{123} reactivity on amino acid content of the proximal histone tail.

The enhanced AP reactivity in NCPs containing mutated histone proteins affirms previous proposals that histone proteins cleave DNA at AP sites via a mechanism (Scheme 1) similar to those employed by base excision repair enzymes charged with the responsibility for excising these lesions from damaged genomes. Lysine residues within the N-terminal histone tails, which are integrally involved in epigenetics, are also key components in this chemistry.^[14] Large amounts of AP sites (10,000 per day per cell) are produced routinely in cells.^[4] Thus, organisms must balance the need for lysines to regulate biochemical events via posttranslational modification with the detrimental effect of catalyzing strand scission at ubiquitous AP sites. Unlike the high abundance of Lys residue, the N-terminal tails of all four histone tails contain only 2 or 3 carboxylate containing amino acids (Glu, Asp) in different organisms from *Saccharomyces cerevisiae* to human (Figure S8). The frequency of Glu and Asp in histone N-terminal amino tails (2–3%) is much lower than that in the proteome (~12%, Table S1). This is expected as the negatively charged Glu and Asp would adversely affect DNA-histone tail binding which regulates many cellular processes. However, this also contributes to the genome's self-preservation by reducing the rate of strand scission at abasic sites. Histidine is even more effective than Glu and Asp at enhancing the strand scission at AP in NCPs by promoting the β -elimination more efficiently. The pK_a of histidine (~6.0) is such that it will exist as a mixture of its neutral and

protonated forms in cells, with the former present in greater amount. Furthermore, histidine phosphorylation is a biologically significant posttranslational modification.^[15] Thus, unlike Asp and Glu, there is no structural reason to minimize His in N-terminal histone tails, but there is a biochemical purpose for including it. However, there are only 2 histidine residues in the N-terminal tails of the 4 histone proteins and histone H4 histidine₁₈ is known to be phosphorylated (Figure S8). In addition, the percentage of His residue in N-terminal histone tails (~1.7%) is significantly lower than its overall presence in the proteome (~2.6%) (Table S1). Therefore, similar to Glu and Asp, histone proteins may also have evolved to contain statistically reduced levels of histidines in their N-terminal tails to protect genomic DNA against self-destruction resulting from the transformation of the most common form of DNA damage, an abasic site, into a single strand break.

Experimental Section

Preparation of histone mutants.

The DNA fragments encoding the *xenopus laevis* H2B mutants (H2B 1–23 Del., H2B 1–23 Del.-K24,25,28,31A, H2B 1–23 Del.-R26,27,30A, H2B 1–23 Del.-R26,27,30E and H2B 1–23 Del.-R26E) were amplified by PCR from pET3a-H2B using the primers listed in Figure S3. The amplified DNA and pET3a plasmid were digested by NdeI and BamHI and the fragments were ligated using the Quick Ligation™ Kit (NEB) to generate the plasmids expressing the H2B mutants. The plasmids expressing the other H2B mutants (H2B 1–23 Del.-R27E, H2B 1–23 Del.-R30E, H2B 1–23 Del.-R27H and H2B 1–23 Del.-R30H) and H4 mutants (H4-H18E and H4-H18D) were generated by site-directed mutagenesis PCR using the pET3a-H2B 1–23 Del. and pET3a-H4 respectively, within the corresponding primers in Figure S3. All the plasmids were purified using a QIAprep Spin Miniprep Kit according to the manufacturer's protocol. The sequences were confirmed by DNA sequencing using the standard T7 primers (Genewiz). The histone proteins were expressed and purified as previously described.^[13] The corrected molecular weights of histone mutants were confirmed by UPLCMS.

Determining the rate constants describing AP disappearance (k_{Dis}) and strand cleavage (k_{Cl}).

NCPs (100 μL , ~1.0 million cpm) with or without NaBH_3CN (10 mM) were photolyzed at 350 nm for 15 min. A portion of the NCPs (20 μL) was removed to determine the fraction of **1** converted to AP. The remainder of the NCPs (80 μL) were incubated at 37°C. Aliquots (10 μL) were removed at appropriate times, immediately frozen in dry ice, and stored at –80°C until the final time point. To determine the fraction of **1** converted to AP, the NCPs (10 μL) were treated with NaOH (100 mM) at 37°C for 30 min, followed by neutralization with HCl and treatment of proteinase K (8 units) at room temperature for 30 min. The samples were analyzed on a 10 % denaturing PAGE gel (20 $\times$ 16 $\times$ 0.1 cm). The gels were run under limiting power (15 W) until the bromophenol blue migrated to the bottom. To determine the products, including DPC_{un} , DPC_{cl} and SSB from AP, the aliquots (10 μL) were treated with fresh NaBH_4 (100 mM) at 4°C for 60 min, followed by mixing with 3 X SDS-loading buffer (5 μL). The samples were analyzed by 10% SDS PAGE (20 $\times$ 16 $\times$ 0.1 cm). The gels were

run under limiting power (7 W) until the bromophenol blue migrated to the bottom. The total amount of reacted AP (AP_{Dis}) as a function of time was calculated using (equation 1).

$$AP_{Dis} = \frac{DPC_{un} + DPC_{Cl} + SSB}{(DPC_{un} + DPC_{Cl} + SSB) \times \text{photolysis efficiency} \times \text{reconstitution efficiency}} \quad (1)$$

The rate constant for AP disappearance (k_{Dis}) was calculated by fitting the remaining AP ($1 - AP_{Dis}$) to a first-order reaction. The amount of reacted AP due to strand cleavage (AP_{Cl}) as a function of time was calculated using (equation 2).

$$AP_{Cl} = \frac{DPC_{Cl} + SSB}{(DPC_{un} + DPC_{Cl} + SSB) \times \text{photolysis efficiency} \times \text{reconstitution efficiency}} \quad (2)$$

The rate constant for strand cleavage (k_{Cl}) was calculated by fitting the remaining AP ($1 - AP_{Cl}$) to a first-order reaction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

- [1]. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ, Nature 1997, 389, 251–260. [PubMed: 9305837]
- [2]. a)Bannister AJ, Kouzarides T, Cell Res. 2011, 21, 381–395; [PubMed: 21321607] b)Dann GP, Liszczak GP, Bagert JD, Müller MM, Nguyen UTT, Wojcik F, Brown ZZ, Bos J, Panchenko T, Pihl R, Pollock SB, Diehl KL, Allis CD, Muir TW, Nature 2017, 548, 607–611; [PubMed: 28767641] c)Brown ZZ, Müller MM, Jain SU, Allis CD, Lewis PW, Muir TW, J. Am. Chem. Soc 2014, 136, 13498–13501. [PubMed: 25180930]
- [3]. a)Ji S, Shao H, Han Q, Seiler CL, Tretyakova NY, Angew. Chem. Int. Ed 2017, 56, 14130–14134;b)Li F, Zhang Y, Bai J, Greenberg MM, Xi Z, Zhou C, J. Am. Chem. Soc 2017, 139, 10617–10620; [PubMed: 28742335] c)Song Q, Cannistraro VJ, Taylor J-S, Nucleic Acids Res. 2014, 42, 13122–13133; [PubMed: 25389265] d)Song Q, Cannistraro VJ, Taylor J-S, J. Biol. Chem 2011, 286, 6329–6335; [PubMed: 21160086] e)Bennett RAO, Swerdlow PS, Povirk LF, Biochemistry 1993, 32, 3188–3195. [PubMed: 7681328]
- [4]. a)Lindahl T, Nature 1993, 362, 709–715; [PubMed: 8469282] b)Lindahl T, Nyberg B, Biochemistry 1972, 11, 3610–3618. [PubMed: 4626532]
- [5]. a)Sczepanski JT, Wong RS, McKnight JN, Bowman GD, Greenberg MM, Proc. Natl. Acad. Sci. U. S. A 2010, 107, 22475–22480; [PubMed: 21149689] b)Zhou C, Greenberg MM, J. Am. Chem. Soc 2012, 134, 8090–8093; [PubMed: 22551239] c)Zhou C, Sczepanski JT, Greenberg MM, J. Am. Chem. Soc 2013, 135, 5274–5277; [PubMed: 23531104] d)Weng L, Greenberg MM, J. Am. Chem. Soc 2015, 137, 11022–11031. [PubMed: 26290445]
- [6]. a)Sczepanski JT, Zhou C, Greenberg MM, Biochemistry 2013, 52, 2157–2164; [PubMed: 23480734] b)Zhou C, Sczepanski JT, Greenberg MM, J. Am. Chem. Soc 2012, 134, 16734–16741. [PubMed: 23020793]

- [7]. a)Matsumoto Y, Kim K, Science 1995, 269, 699–702; [PubMed: 7624801] b)Beard WA, Wilson SH, Chem. Rev 2006, 106, 361–382; [PubMed: 16464010] c)Jacobs AC, Kreller CR, Greenberg MM, Biochemistry 2011, 50, 136–143. [PubMed: 21155533]
- [8]. a)Abbott DW, Gilbert HJ, Borason AB, J. Biol. Chem 2010, 285, 39029–39038; [PubMed: 20851883] b)Jedrzejewski MJ, Mello LV, de Groot BL, Li S, J. Biol. Chem 2002, 277, 28287–28297. [PubMed: 11991948]
- [9]. Parra MA, Kerr D, Fahy D, Pouchnik DJ, Wyrick JJ, Mol. & Cell Biol. 2006, 26, 3842. [PubMed: 16648479]
- [10]. a)Kuduvalli PN, Townsend CA, Tullius T, D., Biochemistry 1995, 34, 3899–3906; [PubMed: 7696253] b)Davey G, Wu B, Dong Y, Surana U, Davey CA, Nucleic Acids Res. 2010, 38, 2081–2088. [PubMed: 20026584]
- [11]. Wang R, Yang K, Banerjee S, Greenberg MM, Biochemistry 2018, 57, 3945–3952. [PubMed: 29894168]
- [12]. Lowary PT, Widom J, J. Mol. Biol 1998, 276, 19–42. [PubMed: 9514715]
- [13]. Dyer PN, Edayathumangalam RS, White CLB, Yunhe, Chakravarthy S, Muthurajan UM, Luger K, Methods Enzymol. 2004, 375, 23–44. [PubMed: 14870657]
- [14]. Huang H, Lin S, Garcia BA, Zhao Y, Chem. Rev 2015, 115, 2376–2418. [PubMed: 25688442]
- [15]. a)Besant PG, Attwood PV, Biochem. Soc. Trans 2012, 40, 290–293; [PubMed: 22260708]
b)Besant PG, Attwood PV, in Meth. Enzymol, Vol. 471, Academic Press, 2010, pp. 403–426. [PubMed: 20946859]

Figure 1. Nucleosome core particle structure. (A.) Overall structure showing AP positioning (B.) Histone H2B residues proximal to AP₁₂₃ (C.) Histone H4 residues proximal to H4 (D.) Sequences of histone H2B (HBR domain highlighted) and H4 N-terminal tails. Structural data are from PDB: 1kx5.

Figure 2. Ratio of DNA-protein crosslinks in the presence of NaBH₃CN (10 mM). (A.) H2B variants in NCPs containing AP₁₂₃ (B.) H4 variants with differences at amino acid 18 in NCPs containing AP₈₉.

Scheme 1.

Strand break formation from AP in a NCP.

Scheme 2.
Photochemical generation of AP.

Table 1.

Abasic site (AP₁₂₃) reactivity in wild type and histone H2B variant containing nucleosome core particles.

Histone H2B	- NaBH ₃ CN	+ NaBH ₃ CN	Rel. rate ^[b]
	$k_{\text{Dis}}[10^6 \text{ s}^{-1}]^{[a]}$	$k_{\text{Dis}}[10^6 \text{ s}^{-1}]^{[a]}$	
Wild type	11.6 ± 1.4	15.2 ± 0.5	1.31
1–23 Del	7.8 ± 0.3	17.8 ± 0.7	2.28
1–23 Del, K24,25,28,31A	1.9 ± 0.2	5.2 ± 1.1	2.73
1–23 Del, R26,27,30A	9.9 ± 0.7	13.5 ± 0.1	1.36
1–23 Del, R26E	31.8 ± 0.4	34.1 ± 1.3	1.07
1–23 Del, R27E	27.2 ± 1.6	29.7 ± 1.9	1.09
1–23 Del, R30E	20.6 ± 1.4	25.5 ± 0.7	1.24
1–23 Del, R26,27,30E	65.3 ± 1.6	56.7 ± 2.4	0.87
1–23 Del, R27H	46.3 ± 6.2	47.9 ± 5.9	1.03
1–23 Del, R30H	29.9 ± 2.3	31.0 ± 3.2	1.04

^[a] Values presented are the average ± s.d. of two experiments, each in triplicate.

^[b] Rel. rate = $[k_{\text{Dis}}(\text{X}) \text{ with NaBH}_3\text{CN}] / [k_{\text{Dis}}(\text{X}) \text{ without NaBH}_3\text{CN}]$, where X corresponds to a particular H2B variant.

Table 2.

Abasic site (AP₈₉) reactivity in wild type and histone H4 variant containing nucleosome core particles.

Histone H4	- NaBH ₃ CN	+ NaBH ₃ CN	Rel. rate ^[b]
	$k_{\text{Dis}}[10^6 \text{ s}^{-1}]^{[a]}$	$k_{\text{Dis}}[10^6 \text{ s}^{-1}]^{[a]}$	
Wild type	25.6 ± 2.6	26.4 ± 3.3	1.03
H18A	7.6 ± 0.1	17.1 ± 1.5	2.24
H18E	16.4 ± 0.3	23.3 ± 0.6	1.42
H18D	16.3 ± 0.7	23.0 ± 1.9	1.41

^[a] Values presented are the average ± s.d. of two experiments, each in triplicate.

^[b] Rel. rate = $[k_{\text{Dis}}(\text{X}) \text{ with NaBH}_3\text{CN}] / [k_{\text{Dis}}(\text{X}) \text{ without NaBH}_3\text{CN}]$, where X corresponds to a particular H4 variant.