Free Radical Biology & Medicine 50 (2011) 749–762

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Factors influencing protein tyrosine nitration—structure-based predictive models

Alexander S. Bayden [a], Vasily A. Yakovlev [b], Paul R. Graves [b], Ross B. Mikkelsen [b,*], Glen E. Kellogg [a,*]

[a] Department of Medicinal Chemistry and Institute for Structural Biology and Drug Discovery, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298, USA
[b] Department of Radiation Oncology, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298, USA

ARTICLE INFO

Article history:
Received 8 October 2010
Revised 15 November 2010
Accepted 10 December 2010
Available online 21 December 2010

Keywords:
Tyrosine nitration
Hydropathic interactions
Oxidative stress
Tyrosyl radical
Free radicals

ABSTRACT

Models for exploring tyrosine nitration in proteins have been created based on 3D structural features of 20 proteins for which high-resolution X-ray crystallographic or NMR data are available and for which nitration of 35 total tyrosines has been experimentally proven under oxidative stress. Factors suggested in previous work to enhance nitration were examined with quantitative structural descriptors. The role of neighboring acidic and basic residues is complex: for the majority of tyrosines that are nitrated the distance to the heteroatom of the closest charged side chain corresponds to the distance needed for suspected nitrating species to form hydrogen bond bridges between the tyrosine and that charged amino acid. This suggests that such bridges play a very important role in tyrosine nitration. Nitration is generally hindered for tyrosines that are buried and for those tyrosines for which there is insufficient space for the nitro group. For in vitro nitration, closed environments with nearby heteroatoms or unsaturated centers that can stabilize radicals are somewhat favored. Four quantitative structure-based models, depending on the conditions of nitration, have been developed for predicting site-specific tyrosine nitration. The best model, relevant for both in vitro and in vivo cases, predicts 30 of 35 tyrosine nitrations (positive predictive value) and has a sensitivity of 60/71 (11 false positives).

Investigation of protein tyrosine nitration has intensified over the past 2 decades, leading to a better understanding of the role of this posttranslational modification in cellular signaling [1]. Although initially considered to be a marker of oxidative stress, there is a growing body of experimental data suggesting that nitration of tyrosine fulfills the criteria of a signal-transducing mechanism [1,2]. For example, tyrosine nitration has been detected under physiological conditions in most organ systems and in a number of cellular models. Furthermore, accumulating data support a strong link between protein tyrosine nitration and the activation of signaling pathways in a variety of cellular responses and pathological conditions, including the cellular response to irradiation, acute and chronic inflammation, graft rejection, chronic hypoxia, tumor vascularization and the microenvironment, atherosclerosis, myocardial infarction, chronic obstructive pulmonary disease, diabetes, Parkinson disease, and Alzheimer disease [3–20].

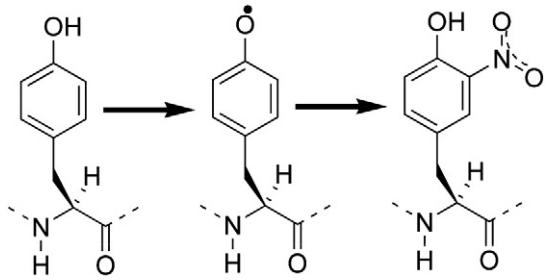
Nitration at tyrosine residues occurs both in vitro and in vivo. Generally, one of the two tyrosine aromatic hydrogens that are *ortho* with respect to the hydroxyl group is replaced by a nitro group. However, in some instances, nitrotyrosine can further react and replace a second hydrogen atom with another nitro group. Most commonly, tyrosine is nitrated posttranslationally in two steps as shown in Scheme 1. First, the tyrosine is oxidized to a tyrosyl radical, which in one of its resonance forms is nitrated in the second step. Several chemical nitrating species have been implicated in tyrosine nitration, including $^{\bullet}NO$, $^{\bullet}NO_2$, $ONOO^-$, $O_2^{\bullet-}$, H_2O_2, NO_2^-, $NO_2CO_3^-$, and $CO_3^{\bullet-}$ [21]. NO_2Cl has also been suggested as a contributor; however, its role in tyrosine nitration in vivo is highly unlikely [22]. Although most evidence suggests that tyrosine nitration occurs posttranslationally, it is formally possible that tyrosine does not have to undergo nitration within the protein. Nitrotyrosine can be transported into cells and then incorporated in proteins during translation [23]. In this case, however, the random incorporation of nitrotyrosine residues into the protein should be observed and this is not the case in vivo.

Tyrosine nitration in proteins does not occur randomly. Most proteins contain tyrosine residues (natural abundance: 3.2%) [24], and tyrosine is often surface-exposed in proteins (only 15% of tyrosine residues are at least 95% buried) and should be easily nitrated [1,25]. However, not all exposed tyrosine residues and not all proteins are nitrated. Neither does the abundance of tyrosine residues in a given protein predict whether it is a target for nitration [1,25,26]. This evidence strongly argues that protein tyrosine nitration is a selective process.

Specific amino acid sequences determine the specificity for other posttranslational modifications involving tyrosine. For example, the peptide sequence surrounding a tyrosine residue contribute to the substrate specificity of tyrosine kinases [27]. An analysis of the primary sequences of proteins nitrated under similar conditions failed to reveal a specific or unique sequence requirement [26].

* Corresponding authors.
E-mail addresses: rmikkels@vcu.edu (R.B. Mikkelsen), glen.kellogg@vcu.edu (G.E. Kellogg).

0891-5849/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2010.12.016



Scheme 1. One mechanism for tyrosine nitration.

Several studies have attempted to determine factors that promote selective tyrosine nitration. It has been reported that tyrosines are more likely to become nitrated when they are in loops [28], which can be expressed as its location with respect to turn-inducing amino acids such as glycine and proline [25]. The role of sulfur-containing amino acids in tyrosine nitration has been debated. Some evidence suggests that these amino acids impede tyrosine nitration [26,29] by competing with tyrosine for nitrating species, whereas others contradict this finding [30], suggesting that sulfur-containing amino acids promote tyrosine nitration. Souza and co-investigators suggest that the presence of acidic residues near the target tyrosine makes it more susceptible to nitration [31]. Lin et al. have shown that when glutamic acid 149 is mutated to alanine in cytochrome P450 2B1, a protein known to be nitrated at tyrosine 190, this nitration is substantially reduced [32]. It has also been shown that tyrosine nitration is facilitated by the presence of a nearby basic amino acid [30] and that the hydrophobicity or hydrophilicity of the environment influences tyrosine nitration [33–35]. Finally, the presence of transition metals seems to encourage nitration of tyrosines [36–39].

Unfortunately, to date there is still no reliable model for predicting tyrosine nitration, as single factors do not satisfactorily explain its selectivity. Other studies have stressed the importance of the protein secondary structure and the local "structural environment" of nitrotyrosine sites [1,25,26]. For example, Gow et al. proposed a mechanism defining specificity of tyrosine nitration that requires consideration of the local environment of tyrosine residues within the secondary and tertiary structures of a protein [2].

In this paper we report the first comprehensive and quantitative investigation of protein structural features that influence tyrosine nitration and in so doing have tested the above anecdotal and qualitative proposals. We have also created an extensive set of additional possible structural factors with potential roles in site-specific tyrosine nitration. Our analysis tested these structural metrics against a training set of known nitrated and (probably) nonnitrated tyrosines; thus, we have built statistical models that can predict tyrosine nitration under different conditions.

Materials and methods

Data set

The nitrated tyrosines we have considered in this work are listed in Table 1. They consist of three overlapping sets: proteins that are nitrated in vitro by chemical means, proteins that are nitrated in vivo by physiological mechanisms, and the union set of all proteins that are nitrated. Thus, results in this work are referenced to these data sets: "in vivo," "in vitro," or "All." For the in vivo and in vitro data sets, the positive controls were tyrosines that have been shown experimentally to be nitrated (see Table 1). For these data sets, we used as negative controls tyrosines in those same proteins that were not reported as nitrated. Additional negative controls available for peroxiredoxin I and porcine aconitase were also used in training our

Table 1
Proteins and residues used in tyrosine nitration study

Protein	PDB code	Nitrated tyrosines	Tyrosines used as nonnitrated controls	Ref.
In vivo nitration				
Muscle creatine kinase	1i0e	14, 20	39, 82, 125, 140, 173, 174, 279	[40]
Actin	1j6z	91, 198, 240	53, 69, 133, 143, 166, 169, 188, 218, 279, 294, 306, 337, 362	[41]
Dihydropyrimidinase-related protein 1	1kcx	316[a]	32, 36, 75, 135, 145, 167, 170, 174, 182, 251, 290, 336, 395, 431, 479	[30]
IκBα (monomeric)	1ikn	181[a]	195, 248, 251, 254, 289	[42]
Calcineurin	1m63	224	113, 119, 124, 132, 140, 159, 170, 175, 258, 260, 262, 288, 291, 311, 315, 324, 341	[30]
Mitochondrial creatine kinase	1qk1	274	9, 15, 34, 77, 95, 115, 120, 168, 169, 354	[30]
Clathrin adaptor protein complex 1	1w63	574[a]	6, 70, 72, 76, 121, 136, 229, 276, 277, 300, 328, 333, 361, 405, 421, 425, 455, 524, 526, 566	[30]
Kelch-like ECH-associated protein 1	1u6d	345, 491, 537	329, 334, 341, 375, 396, 426, 443, 473, 490, 520, 525, 567, 572, 584	[43]
Escherichia coli ribonucleotide reductase protein R2	1rib	122[a], 273, 289	2, 28, 33, 62, 79, 156, 157, 166, 194, 209, 307, 310	[44]
Muscle glycogen phosphorylase	2amv	113, 161	51, 52, 83, 84, 185, 203, 226, 233, 262, 280, 297, 374, 404, 472, 511, 524, 548, 553, 587, 613, 726, 731, 732, 777, 780, 791, 820	[40]
14-3-3 β	2bq0	84	21, 50, 106, 120, 127, 130, 151, 180, 181, 213	[30]
p53 (tetramerization domain)[b]	2j0c	327	None	[45]
Triosephosphate isomerase	2jk2	67[a], 208	47, 164	[30]
Brain-type creatine kinase, B chain	3b6r	269	39, 68, 82, 100, 125, 173, 174, 279	[30]
Fructose-1,6-bisphosphate aldolase	3bv4	173, 203	58, 84, 137, 213, 222, 243, 301, 327, 342	[30,43]
In vitro nitrations				
p53 (DNA-binding domain)	2fej	107	103, 126, 163, 205, 220, 234, 236	[45]
Bovine ribonuclease A	1jvt	115[a]	25, 73,76, 92, 97	[25]
Bovine Cu Zn SOD	1e9q	108	None	[46]
Peroxiredoxin I	2rii	None	116	[46]
Actin	1j6z	53, 198, 240, 362	69, 91, 133, 143, 166, 169, 188, 218, 279, 294, 306, 337	[44]
E. coli ribonucleotide reductase protein R2	1rib	62, 122[a]	2, 28, 33, 79, 156, 157, 166, 194, 209, 273, 289, 307, 310	[47]
Muscle creatine kinase	1i0e	82	14, 20, 39, 125, 140, 173, 174, 279	[43]
Bacteriorhodopsin	1brd	26	43, 57, 79, 83, 147, 150, 185	[48]
Porcine aconitase	1boj	None	43, 60, 74, 96, 136, 178, 206, 223, 268, 274, 301, 306	[49]
GST-1	2h8a	92	18, 115, 120, 137, 145	[50]
Lysozyme	2zxs	20, 23	53	[25]

PDB, Protein Data Bank.
[a] These tyrosine environments were represented in model training by single subunits although there are potentially interacting residues in other subunits of that protein.
[b] Experimental evidence suggests that p53 is nitrated in its monomeric form. This protein/residue was not used in training, but only in testing the model.