TiO₂ Nanoparticles Catalyze Oxidation of Huntingtin Exon 1-Derived Peptides Impeding Aggregation: A Quantitative NMR Study of Binding and Kinetics

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Supporting Information

ABSTRACT: Polyglutamine expansion within the N-terminal region of the huntingtin protein results in the formation of intracellular aggregates responsible for Huntington’s disease, a fatal neurodegenerative condition. The interaction between TiO₂ nanoparticles and huntingtin peptides comprising the N-terminal amphiphilic domain without (httNT) or with (httNTQ10) a ten-residue C-terminal polyglutamine tract, is investigated by NMR spectroscopy. TiO₂ nanoparticles decrease aggregation of httNTQ10 by catalyzing the oxidation of Met7 to a sulfoxide, resulting in an aggregation-incompetent peptide. The oxidation agent is hydrogen peroxide generated on the surface of the TiO₂ nanoparticles either by UV irradiation or at low steady-state levels in the dark. The binding kinetics of nonaggregating httNT to TiO₂ nanoparticles is characterized by quantitative analysis of ¹⁵N dark state exchange saturation transfer and lifetime broadening NMR data. Binding involves a sparsely populated intermediate that experiences hindered rotational diffusion relative to the free state. Catalysis of methionine oxidation within the N-terminal domain of the huntingtin protein may potentially provide a strategy for delaying the onset of Huntington’s disease.

Polyglutamine expansion within the N-terminal region of the huntingtin protein (corresponding to exon-1) favors aggregation and is responsible for Huntington’s disease, a fatal neurodegenerative condition.¹ The polyglutamine domain lies downstream of the 16-residue N-terminal amphiphilic domain (httNT). Peptides comprising httNT with as few as 10 glutamines (httNTQ₁₀) aggregate rapidly in solution and form polymorphic fibrils.² We recently observed that oxidation of the Met7 side-chain to a sulfoxide (Met7O) prevents aggregation of httNTQ₁₀.³ In general, adsorption of fibril forming proteins and peptides on the surface of nanoparticles enhances aggregation and fibril formation by increasing the local peptide concentration and hence the probability of forming a critical initiation nucleus.⁴ TiO₂ nanoparticles (TiO₂ NPs) are unique as they possess photocatalytic properties that generate reactive oxygen species upon UV irradiation.⁵ Here we study the interaction of httNT and httNTQ₁₀ with TiO₂ NPs by NMR, and show that TiO₂ NPs specifically catalyze the oxidation of Met7, thereby preventing fibril formation by reducing the concentration of the aggregation-competent, native reduced form of httNTQ₁₀.

Spontaneous aggregation of httNTQ₁₀ occurs over time as monitored both by an increase in Thioflavin T (ThT) fluorescence emission (Figure 1A), attributed to the formation of β-rich amyloid-like structures, and by a decrease in the intensity of the amide proton envelope of the NMR signal (Figure 1B; see SI). Reduced and oxidized monomeric httNTQ₁₀ are NMR visible, while aggregates of reduced httNTQ₁₀ are broadened beyond detection, resulting in a decrease in the observable amide proton envelope intensity. Addition of photoexcited TiO₂ NPs (see SI and Figure S1) dramatically reduces the extent of aggregation. At 10 °C, only 8% of a 300 μM ¹⁵N-httNTQ₁₀ sample remains NMR visible after 100 h; in the presence of photoexcited TiO₂ NPs, however, aggregation plateaus out with 33% of the sample remaining NMR visible (Figure 1B). The apparent aggregation t₁/₂ (~23–25 h), however, is not affected by the TiO₂ NPs (Figure 1B). The reduction in the fraction of aggregating...
species can be attributed to TiO2 NP catalyzed oxidation of the side chain of Met7 to a sulfoxide. Several reactive oxygen species are formed on the surface of TiO2 NPs upon UV irradiation (Figure S2), but only H2O2 is stable with significant amounts generated both upon UV irradiation and in the dark (Figures 2A and S3).

The kinetic and mechanistic details of15N-labeled httNT adsorption on the surface of TiO2 NPs for many days (Figure S4). Oxidation of httNT by H2O2 free in solution makes a significant contribution to the kinetics of httNT oxidation in the presence of 5 g L⁻¹ TiO2 NPs as the concentration of dissolved H2O2 generated upon UV irradiation (~11 μM, Figure 2A) is 10-fold lower than that used in the experiment with added H2O2 shown in Figure 2B. The time course of oxidation in the presence of UV irradiated TiO2 is biphasic (Figure 2C). The first phase arises from second-order (k2 ~ 500 M⁻¹ h⁻¹) oxidation of Met7 by the substantial amount of H2O2 located on the surface of the TiO2 NPs generated by UV irradiation. Oxidation on the TiO2 NP surface is accelerated ~45-fold relative to that free in solution. Once H2O2 generated by UV irradiation is consumed, a slower apparent first order oxidation process (k3 dark = k3[H2O2]dark ~ 0.002 h⁻¹) occurs owing to the steady-state level of H2O2 present on the TiO2 NP surface in the dark. With these values of k2 and k3 dark, Scheme 1 quantitatively accounts for the disappearance of NMR visible httNTQ10 in the presence of photoactivated TiO2 NPs with kagg ~ 0.03 h⁻¹ which remains unchanged in the absence of TiO2 NPs (Figure 1B).

The efficiency of heterogeneous catalysis is dependent upon the strength of interaction between the adsorbate (httNT) and the surface of the catalyst. We therefore characterized the kinetic and mechanistic details of15N-labeled httNT adsorption on the surface of TiO2 NPs using 15N dark state exchange saturation transfer (DEST) and lifetime line broadening (ΔR) which enable one to quantitatively analyze exchange processes between an NMR visible species and very high molecular weight, NMR invisible “dark” states. Although binding of httNTQ10 to TiO2 NPs cannot be studied quantitatively owing to the hight NTQ10 aggregation during the time course of the DEST experiment (several days), ΔR measurements are sufficiently fast (a few hours) to demonstrate significant 15N lifetime line broadening (~10 s⁻¹), and hence binding, of httNTQ10 in the presence of TiO2 NPs (Figure S5).

Scheme 1. Parallel Reactions Describing Aggregation and TiO2 NP-Catalyzed Oxidation of httNTQ Peptides

To study the kinetics of TiO2 catalyzed oxidation of huntingtin peptides in the absence of aggregation (kagg = 0 in Scheme 1), we made use of a peptide comprising only the N-terminal amphiphilic domain, httNT, which remains stable in the presence of TiO2 NPs for many days (Figure S4).

Figure 2. TiO2-catalyzed oxidation of httNT. (A) Amplex Red assay for H2O2 generated by 5 g L⁻¹ TiO2 NPs in the dark (blue, ∼4 μM H2O2) and upon UV exposure for 3 h (red, NP suspension, ∼76 μM H2O2; green, supernatant after removal of NPs, ∼11 μM H2O2). (B) Time course of Met7 oxidation of 300 μM 15N-labeled httNT following addition of (B) 100 μM H2O2 and (C) UV-irradiated TiO2 NPs (5 g L⁻¹, 3 h UV exposure) monitored by the reduction and corresponding increase in intensities of Ala1 and Phe10 cross-peaks arising from reduced (blue) and Met7O oxidized (red) httNT, respectively, in a series of 1H−15N HSQC spectra. The insets in (C) show the cross-peaks corresponding to the reduced (upfield) and oxidized (downfield) states at t = 0 (purple) and 80 (green) hours. The experimental data in panels B and C are shown as circles and the best-fit curves obtained by nonlinear optimization and integration of the differential equations (eq S1) describing the oxidation process in Scheme 1 are represented by solid lines. The differential ratios of oxidized to reduced httNT at time zero in panels (B) and (C) reflect sample to sample variations. Data were collected at 10 °C and a spectrometer frequency of 600 MHz.

The oxidation kinetics of httNTQn huntingtin peptides (where n = number of glutamines in the polyglutamine tract) in the presence of TiO2 NPs can be described by three parallel reactions (Scheme 1): two second-order processes involving oxidation of httNTQn (Pred) to Met7-O-httNTQn (Poxi) by H2O2 generated upon UV irradiation, either dissolved in solution (k1) or on the surface of the TiO2 NPs (k2), and a pseudo-first-order process (k3 dark = k3[H2O2]dark) occurring in the dark that involves a low steady-state (i.e., continuously generated) level of H2O2 on the surface of the TiO2 NPs. These reactions occur concomitantly with aggregation of Poxi which, for simplicity, is described as a unimolecular process with a rate constant kagg. Our treatment of the kinetics of all oxidative processes assumes that oxidation proceeds on a timescale much slower than that of binding to TiO2 NPs, as is amply confirmed experimentally (see below).
The binding of Met 7O-httNT to TiO2 NPs was also characterized by analysis of 15N-DEST and ΔR2 data (Figure S6). The population of the bound state \( \text{P}_{\text{B}}^{\text{red}} \) is \( \sim 0.6\% \), ~3-fold lower than that of the reduced form, indicating that oxidation of Met’ reduces the binding affinity to TiO2 NPs, in agreement with previous studies on the interaction of httNTQ2 with lipid micelles.3

In conclusion, the current work provides a mechanistic basis for understanding the interaction of huntingtin peptides with TiO2 NPs coupled with surface-catalyzed oxidation, and suggests that targeted catalysis of Met’ oxidation within the httNT domain of the huntingtin protein may provide a strategy for delaying the onset of Huntington’s disease.

ASSOCIATED CONTENT

Supporting Information
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Experimental details, fitting procedures and three additional figures (PDF)

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Notes

The authors declare no competing financial interest.
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