What can be learned about biochemical reactions from neutron diffraction studies?

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Abstract

The precise locations of hydrogen atoms are important for an understanding of the catalytic mechanism of many enzymes. Neutron diffraction provides an excellent experimental method for directly determining the positions of hydrogen atoms in proteins. Neutron diffraction can also provide information on hydrogen bonding networks between water molecules and protein side chains. We have determined the neutron diffraction structure of the enzyme D-xylose isomerase whose mechanism of action contains a ring-opening step and an isomerization step. Both of these steps involve hydrogen atoms. We present examples of hydrogen atom locations on functional groups such as histidine and lysine, in addition to hydrogen-bonding networks found at the active site of this enzyme.

Introduction

Neutron diffraction studies of macromolecules can be used to locate hydrogen atom positions at modest resolutions (1.5-2.4 Å). The hydrogen isotope, deuterium, scatters neutrons to the same extent as do carbon, nitrogen, and oxygen and better than many metal ions. In addition, deuterium scattering gives a positive peak in the neutron-density map, while the more common isotopic hydrogen atom will give a negative peak in such a map. Therefore, for deuterated species, neutron-density maps may contain very different useful information from that in electron-density maps. For example, neutron diffraction studies may indicate which are the more mobile and labile hydrogen atoms. In addition, neutron diffraction studies can reveal the protonation states of amino acid residues in the active site, together with hydrogen atom locations in hydrogen bonds. We will use the neutron structure of the enzyme D-xylose isomerase (which catalyzes the interconversion of D-xylose and D-xylulose or D-glucose and D-fructose) to illustrate specific features related to biochemical reactions and protein structure.

Results

Role of Histidine in Enzymatic Reactions

In the elucidation of enzyme reaction mechanisms, it is essential that the protonation states of active site amino acid residues be determined. For example, the identification of the protonation state of amino acids in the catalytic center of the serine protease trypsin by neutron diffraction helped solve the question of which of the two amino acids, Asp-102 or His-57, is the origin of the catalytic proton. Trypsin contains a catalytic triad composed of His-57, Asp-102 and Ser-195. Neutron diffraction studies of the deuterated bovine enzyme-inhibitor complex at a pH near 7, clearly showed (at 2.2 Å resolution) that there were two nitrogen-bound deuterium atoms on His-57 and none on Asp-102.

D-xylose isomerase contains a triad consisting of His-54, Asp-57 and D-xylose or D-glucose. Our neutron structure of the native unliganded form indicates that His-54 is protonated at both ND1 and NE2. The proton located on ND1 forms a hydrogen bond to OD2 of Asp-57 and the proton located on NE2 hydrogen bonds to the oxygen atom of W1022. This water molecule is displaced when substrate (D-xylose or D-glucose) is bound (Figure 2b). A hydrogen bond is formed by NE2 to the ring oxygen of the substrate, initiating ring opening of the cyclic sugar to give its linear form. The protonation states of all histidines in D-xylose isomerase are shown in Tables 1 and 2 from both our neutron and ultra-high (0.94 Å) resolution X-ray structure.

Table 1. Singly protonated (proton located on either ND1 or NE2)

His	ND1 to	%D	NE2 to	%D	ND1 B	NE2 B	ND1 e.d.	NE2 e.d.	Comments	Inference	
	neutron				X ray					ND1	NE2
49	Pro-7 O	37	-	-	15.8	14.4	2 sigma	none		D	none
71	W1204 (D2)	0	W1281	46	18.9	19.8	none	none	probable disorder	none	D
96	Val-98 N	0	W1210	32	10.8	13.3	none	1.5 sigma	amide D to ND1	none	D

Table 2. Doubly protonated (both ND1 and NE2 are protonated)

His	ND1 to	%D	NE2 to	%D	ND1 B	NE2 B	ND1 e.d.	NE2 e.d.	Inference	
	neutron				X ray				ND1	NE2
54	Asp-57	67	W1022	50	10.6	10.7	2 sigma	none	D	D
198	Thr-195 OG1	54	W1023	52	8.4	9.2	2 sigma	2 sigma	D	D
220	Pro-182 O	64	Metal Ion	57	14.4	17.7	none	none	D	D
230	W1065	67	W1214	87	8.9	9.5	2 sigma	none	D	D
243	Asn-215 OD1	91	W1026	32	13.2	14.1	2 sigma	none	D	D
285	Asp-245	100	Thr-52 OG1	34	10.3	10.4	2 sigma	none	D	D
382	W1109	69	Asp-323	49	10.6	10.7	none	none	D	D

Histidine and lysine protonation states from neutron diffraction studies of the enzyme D-xylose isomerase at 1.8 Å resolution

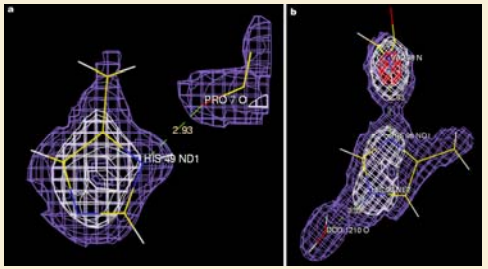

Figure 1. (a) His-49 is singly protonated, at ND1. (b) His-96 is singly protonated at NE2. Note the hydrogen bond from Val-98 N-H to His-96 ND1, which is not protonated.

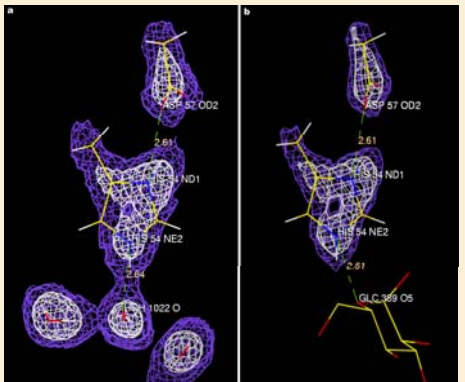

Figure 2. Neutron structures. (a) His-54 is doubly protonated. ND1 forms a hydrogen bond to Asp-57 OD2. NE2 forms a hydrogen bond to W1022. (b) His-54 neutron density. Superimposed on this map is alpha-glucose from 1XIF (X-ray structure); this replaces W1022 with the glucose ring oxygen at a hydrogen bonding distance from NE2. Ring opening of the glucose is thought to be initiated in this way.

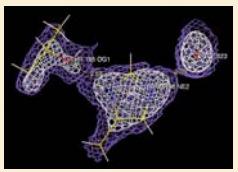

Figure 3. His-198 is doubly protonated. ND1 forms a hydrogen bond to OG1 of Thr-195. NE2 forms a hydrogen bond to W1023. See Table 2.

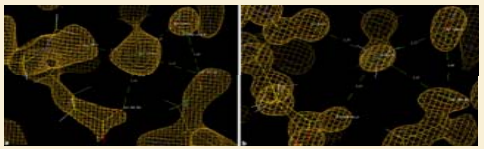

Figure 4. (a) The nuclear density of Lys-183 defines (at 2σ) the orientation of the terminal ND_3^+ group. This results in hydrogen bonds to the main chain oxygen atom of Glu-186, to a carboxylate oxygen atom Glu-186 OE1, and to W1165. (b) On the other hand the X-ray electron density map (0.94 Å) of Lys-183 does not indicate the orientation of the terminal ND_3^+ group.

Neutron diffraction reveals water structure

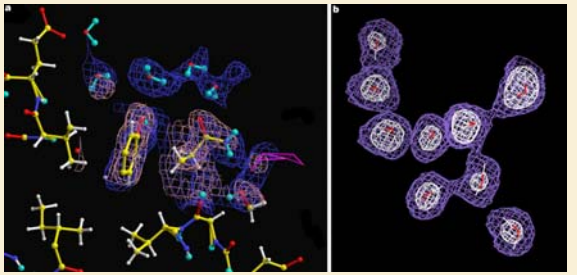

Figure 5. D-xylose isomerase. (a) Superposition of electron density from 1XIB ('coral') and neutron density (blue). Only one of the four water molecules in the upper central region was identified in the X-ray structure. The electron density was calculated with the weighted coefficients, 2mFo-DFcalc, from Refmac5 at 1.6 Å resolution. Both the X-ray and neutron density are displayed at a contour level of 1.5σ. (b) The orientation of the protons in water molecules can often be determined from neutron density, as illustrated in a different string of water molecules shown in the figure.

Conclusions

Information that can be obtained from neutron diffraction studies of macromolecules:

1. Protonation states of active-site amino acid residues that might play a role in the catalytic mechanism are revealed. We have shown here how well this works for histidine and lysine.
2. Not all of the hydrogen atoms of interest are visible in the X-ray derived (ultra-high resolution, 0.94 Å) electron-density map of D-xylose isomerase.
3. Our 1.8 Å neutron structure of D-xylose isomerase clearly shows a proton located at both the ND1 and NE2 sites of His-54. The transfer of this proton to O5 of the cyclic substrate sugar is thought to initiate ring opening to give a linear sugar. This is the first time this proton has been experimentally observed.
4. Neutron diffraction studies may also indicate which are the more labile and mobile hydrogen atoms. Because of the differences in the scattering power of hydrogen and deuterium it is possible to determine to what extent they exchange in a crystal structure that has been soaked in D_2O. Such results provide information on the rigidity and flexibility of interatomic interactions in the structure.
5. Neutron diffraction studies provide a means to evaluate the protonation states of amino acid residues at various pH values. This can be relevant to an understanding of the mechanism of action of the enzyme.
6. Protein-water interactions shown in the neutron density map above highlight the importance of water for folding and stabilization of protein structures. Such interactions provide a channel for substrate and product access to and from the active site. The chemistry of water interacting with protein main chain and side chains is not so amenable from X-ray structures as it is from neutron structures.

References

Carrell, H. L., Hoier, H. & Glusker, J. P. (1994) *Acta Cryst.* D50, 113-123.
Carrell, H. L., Glusker, J. P., Burger, V., Manfre, F., Tritsch, D. & Biellmann, J.-F. (1989) *Proc. Natl. Acad. Sci. USA* 86, 4440-4444.
Collyer, C. A., Henrick, K. & Blow, D. M. (1990a) *J. Mol. Biol.* 212, 211-235.
Collyer, C. A. & Blow, D. A. (1990b) *Proc. Natl. Acad. Sci. USA* 87, 1362-1366.
Emsley, P. & Cowtan, K. (2004) *Acta Cryst.* D 60, 2126-2132.
Hanson, B. L., Langan, P., Katz, A., Li, X., Harp, J. M., Glusker, J. P., Schoenborn, B. P. & Bunick, G. J. (2004) *Acta Cryst* D 60, 241-249.
Kossiakoff, A. A. & Spencer, S. A. (1980) *Nature* 288, 414-416.
McRee, D.E. (1999) *J. Struct. Biol.* 125, 156-156.
Whitlow, M., Howard, A. J., Finzel, B. C., Poulos, T. L., Winborne, E. & Gilliland, G. L. (1991) *Proteins, Struct. Funct. Genet.* 9, 153-173.

Work supported by NIH grants CA-10925, CA-06927, GM-29818 and NASA NAG8-1826, the Office of Science, the Office of Biological and Environmental Research of the US Department of Energy.