How Polyubiquitin Chains are Made and Unmade

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Intermediates in Ub Activation by E1

The Sequence and Distribution of Enzyme Intermediates
(Haas & Rose, 1982)
How a protein may become polyubiquinate?

(Alberts, The Cell, p. 360)
Test of E1 as a Continuous Ub Source

$$E1.AMP-\text{Ub}^* . S-\text{Ub}^*$$  \hspace{1cm} (PULSE)

$$\downarrow$$

$$E2.E3.\text{Protein} + \text{ATP} + \text{Ub}$$  \hspace{1cm} (CHASE)

$$\downarrow$$

$$\text{Protein} - \text{Ub} - \text{Ub}^* - \text{Ub}^*$$

if $E1.AMP-\text{Ub} \rightarrow ES-\text{Ub}$ is fast

or $\text{Protein} - \text{Ub} - \text{Ub} - \text{Ub}^*$

if $E3.\text{Protein}$ dissociates faster
High mw conjugates formed with labeled Ub in reticulocyte extracts on G75 (A ) are shown to breakdown to Ub if ATP is withdrawn as in B as shown by their regeneration when ATP is added to (B ) (Hershko, et al, PNAS, 1980)
Ubiquitin Carboxy-terminal Hydrolase

Ub + E$_1$SH $\rightarrow$ ES Ub $\rightarrow$ GS-Ub

ATP $\rightarrow$ AMP + PPi

GSH (Spon.) $\rightarrow$ GSH transthiolation

(New Enzyme) $\rightarrow$ GSH
Inactivation of UCH by NaBH₄

\[
E - SH \quad + \quad Ub \quad \leftrightarrow \quad E - S - C - Ub_{75}
\]

\[
\text{NaBH₄} \quad \downarrow \quad \text{OH}
\]

\[
E - S - C - Ub_{75} \quad \downarrow \quad H
\]

\[
\text{(inactive UCH)}
\]

\[
\text{H} \quad \triangleleft \quad \text{acid}
\]

\[
O = C - Ub_{75} \quad \text{(Ubaldehyde)}
\]

1. Free Ubal + NaBH₄ → Ub₇₅-ethanolamine
2. Ubal binds UCH 1000x tighter than Ub
   (Pickart and Rose, 1986)
Q: Why is Ub a good inhibitor?