New Findings about Ellman's Method to Determine Cholinesterase Activity

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The original Ellman's spectrophotometrical method for cholinesterase activity determination uses 5,5′-dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) as a chromogen and records the level of cholinesterase activity as an increase of absorbance at 412 nm. Although this procedure usually poses no problem, exceptions arise when the concentration of DTNB is far higher than the concentration of acetylthiocholine (ATCH). It was found that the ratio of concentrations of DTNB/ATCH is an important parameter for the ATCH hydrolysis course: high excess of DTNB decreases the hydrolysis rate resulting in a lower measured enzyme activity. Our experiments indicate that this influence of DTNB concentration can be explained by the inhibition of ATCH hydrolysis by DTNB.

Key words: Ellman's Method, Acetylthiocholine, Hydrolysis