Chinese Chemical Letters Vol. 16, No. 10, pp 1335-1336, 2005 1335
http://www.imm.ac.cn/journal/ccl.html

Chemical Modification of Tryptophan Residues in Pullulanase

Li Rong TENG, Hao FAN, Yuan Yuan ZHANG, Qi YU,
Yue Feng HUANG, Lan Ying LIU*

College of Life Science, Jilin University, Changchun 130023

Abstract: Tryptophan(Trp)residues in pullulanase have been chemically modified with N-bromo-ssuccinimide(NBS). The results of ultraviolet spectra indicated that there are 18 Trp residues in pullulanase and nine of them are located on the surface of the enzyme. Three of these Trp residues are none-essential residues which showed the fastest reaction speed by Zhou's plot. Two of the seven relative faster reacting residues are essential for the activity of the enzyme. The other eight are none-reactive residues with lowest reaction speed.

Keywords: Pullulanase, tryptophan(Trp), chemical modification.

Pullulanases (E.C.3.2.1.41) are debranching enzymes which can hydrolyze α-1,6 glucosidic bond in pullulan, amylopectin and β-limit dextrin and so on[1,2]. His 607, Asp 677, His 833 were determined to locate in the active site of pullulanase[3], but there are not many reports on essential groups, which are not located in the active site, especially on tryptophan. In this study, we investigated the distribution of tryptophan residues and their effects on the biological function of pullulanase using N-bromosuccinimide (NBS) as the modification agent, in order to provide information for further studies on the structure and function of pullulanase.

In order to modify all the Trps in the molecule of pullulanase, the enzyme was completely denatured by adding 8 mol/L urea solution and boiled for 5 min. Then the denatured enzyme (0.22 mg/mL) was modified by different volumes of NBS solution (1.7 mmol/L) and the ultraviolet absorbtion was determined at the wavelength of 280 nm. The number of Trp residues oxidated was calculated according to the Spande spectrophotometry in ref. 4 as follows:

$$n = \frac{\Delta A \times 1.31 \times Mw \times V}{W \times 5500}$$

where n stands for the mole number for Trp residues oxidated by NBS per mol enzyme, ΔA stands for the difference of absorbency between control solution and enzyme solution oxidated by NBS, 1.31 stands for the Witkop factor, Mw stands for the molecular weight of pullulanase, V stands for the volume of enzyme solution, W stands for the weight of pullulanase, and 5500 stands for the molar extinction coefficient of Trp at the wavelength of 280 nm. When 60 μL NBS solution was added, the $A_{280\ nm}$ of denatured enzyme

* E-mail: ying@public.cc.jl.cn

Figure 1 Relationship between number of modified Trp residues and residual activity

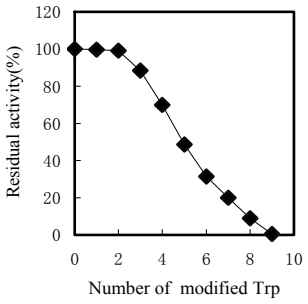
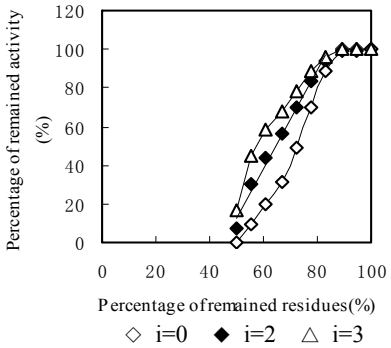


Figure 2 Zou's plot of modified pullulanase



reached the nadir. It was calculated that there are eighteen Trp residues in one pullulanase molecule based on the equation described above.

The number of Trp residues in natural pullulanase was determined and calculated according to the method described above. When 30 μL NBS solution was added the $A_{280\,nm}$ of natural enzyme reached the nadir. It indicated that there are nine Trp residues on the surface of one pullulanase molecule, while the other nine are located inside the enzyme.

Quantity relationship between the modification of Trp residues and the activity of pullulanase were determined. The activity of enzyme without NBS was used as the 100% and the result was shown in **Figure 1**.

In order to identify the reacting properties of Trp residues respectively, Zou's plot from ref. 5 and the following formula was applied:

$$a^{1/i} = \frac{nx-(n-p-s)}{p}$$

where a stands for the remained percentage of activity, x stands for the Trp residues remaining percentage, n stands for the sum of Trp residues, s stands for the number of fastest none-essential residues, p stands for the faster residues, and i stands for the essential residues among the faster residues. The linear relationship between $a^{1/i}$ and x could be basically achieved when i is equal to two (**Figure 2**). It can be calculated from the graph that n/p =2.54, (n-p-s)/p=1.14. p was determined approximately to be seven while s to be three. The results indicated that three out of the eighteen Trp residue are reacting fastest but none-essential. Two of the seven residues are reacting rather fast which are essential for the activity of the enzyme. The other eight are reacting the slowest or none-reacting at all.

References

1. G. D. Albertson, R. H. McHale, *Biochimica et Biophysica Acta* [J], **1997**, *1354*(1), 35.
2. G. Kriegshauser, W. Liebl, *Journal of Chromatography B* [J], **2000**, *737*(1-2), 245.
3. M. Yamashita, D. Matsumoto, Y. Murooka, *J Ferment Bioeng* [J], **1997**, *84*(4), 283.
4. T. F. Spanda, B. Witkop, *Methods in Enzymology* [J], **1967**, *11*, 496.
5. C. L. Zhou, *Journal of Biochemistry and Biophysics* [J], **1962,** *2*(3), 203.

Received 29 December, 2004