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A Simple Activity Measurement of Lysozyme

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Short Communication

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A Simple Activity Measurement of Lysozyme

Sir:

Bacterial cell wall lysis has been widely employed as a measure of the activity of lysozyme. Because of the complicated nature of this assay, it does not always reflect the true enzymic reaction.[1] Methods which use oligo or poly N-acetylglucosamine (NAG*) derivatives as the substrate have been developed recently. These assays were monitored by viscosimetry[2,3] or reducing group analyses.[4,5] This report describes a simple and reproducible reducing group analysis method by a modification of Schales' procedure.[6]

The color reagent solution was made by dissolving 0.5 g potassium fericyanide in 1 litter of 0.5 M sodium carbonate and stored in a brown bottle. Two ml of the reagent were mixed with 1.5 ml of sample solution and incubated in boiling water for 15 min in a test tube stoppered with aluminum foil. After cooling, the optical density at 420 nm was read versus water. The concentration of sodium carbonate was sufficiently high so as not to be affected by the buffer action of the sample solution. The color development was not affected by the high concentration of sodium carbonate. The color was also not affected when the mixture was allowed to stand for a few hours before or after boiling or when the boiling period was varied from 10 to 30 min as long as NAG and $(NAG)_2$ were used. Thus a stable and reproducible color is developed. A blank value of 0.850 ($O.D._{420}$) was obtained within $\pm 1\%$ deviation.

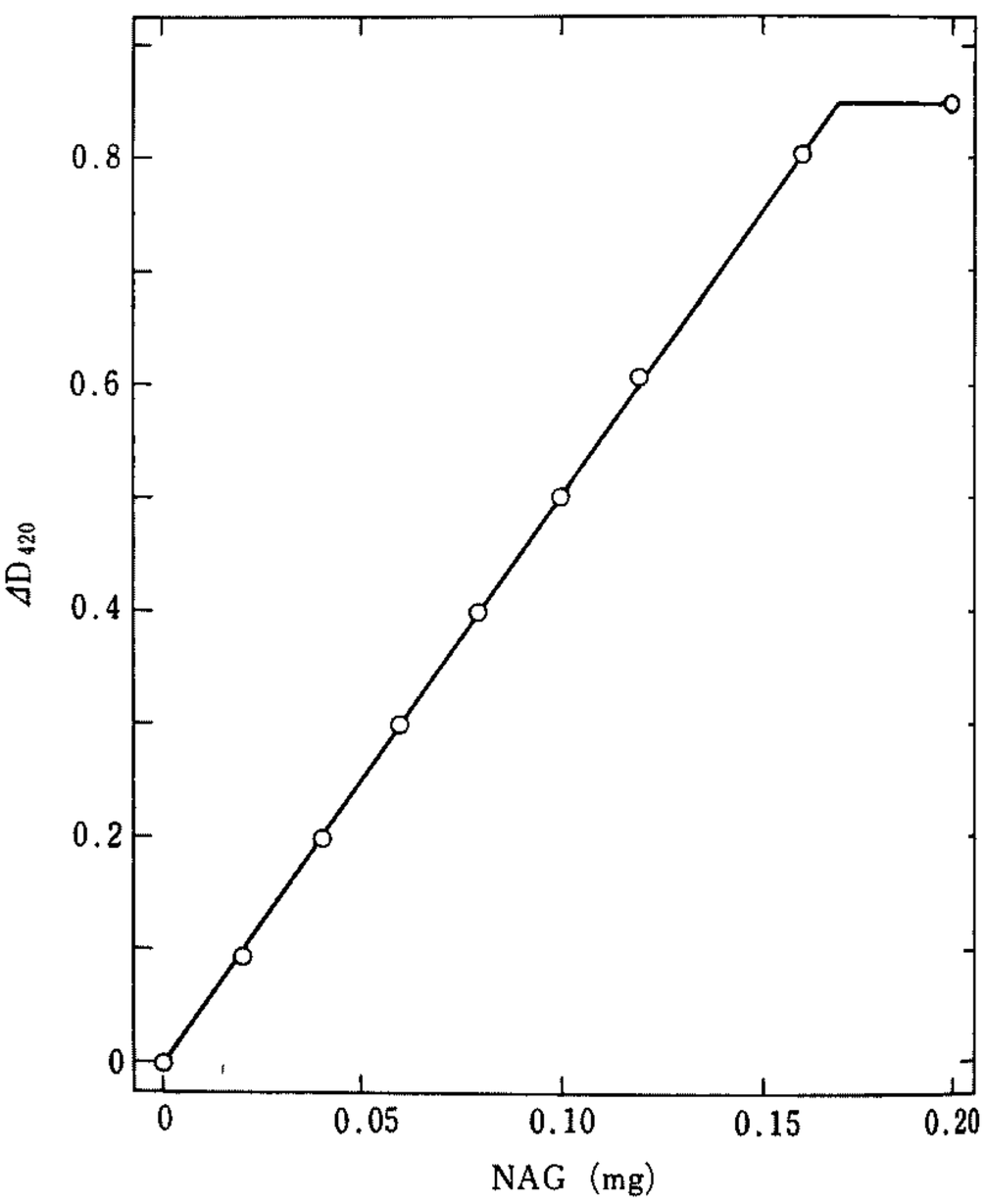

FIG. 1. Standard Curve for N-Acetylglucosamine.

The mixture of 2 ml of the color reagent and 1.5 ml of NAG solution was incubated in boiling water for 15 min and the decrease in $O.D._{420}$ was plotted against NAG concentration.

A standard curve for NAG is shown in Fig. 1. The change in optical density is directly proportional to the NAG concentration and very

* Abbreviations used are: NAG; N-acetylglucosamine, NBS; N-bromosuccinimide.

1) N. Yamasaki, K. Hayashi and M. Funatsu, *Agr. Biol. Chem.*, **32**, 64 (1968).

2) K. Hamaguchi and M. Funatsu, *J. Biochem.*, **46**, 1659 (1959).

3) A. Marzottl and L. Galzigna, *Hoppe-Seyler's Z. Phys. Chem.*, **350**, 427 (1969).

4) K. Hayashi, N. Yamasaki and M. Funatsu, *Agr. Biol. Chem.*, **28**, 517 (1964).

5) M. Imanishi, N. Miyagawa and T. Amano, *Biken J.*, **12**, 25 (1965).

6) O. Schales and S. S. Schales, *Arch. Biochem.*, **8**, 285 (1945).

reproducible. The decrease in total color value ($\Delta D_{420} \times$ml) was constant (18.0±0.5/mg NAG) for a given NAG amount in several fericyanide concentrations at least upto half the concentration employed here. The curve shows that 0~0.15 mg of NAG can be detected under these conditions (caution must be taken with other oligomers which have different color values). This method has adequate sensitivity for the detection of reducing groups produced by lysozyme action. Park-Johnson's method[7] is too sensitive (affected by most cations present) and Somogyi's method[8] is not sensitive enough.

For the measurement of lysozyme, glycol chitin[9] was used as a substrate. To 1 ml of 0.05% glycol chitin solution, 0.5 ml of lysozyme solution was added and incubated for 30 min at 40°C. Both solutions were buffered in 0.1 M acetate at pH 4.5 and preincubated at 40°C. After the reaction, 2 ml of color reagent were added and the mixture was immediately boiled for 15 min (a slight lysozyme reaction was detected even after addition of the color reagent). The glycol chitin solution itself did not cause any decrease in color. This means that glycol chitin used had a high degree of polymerization with a low content of reducing groups, and that non-specific hydrolysis of glycol chitin did not take place under the assay conditions.

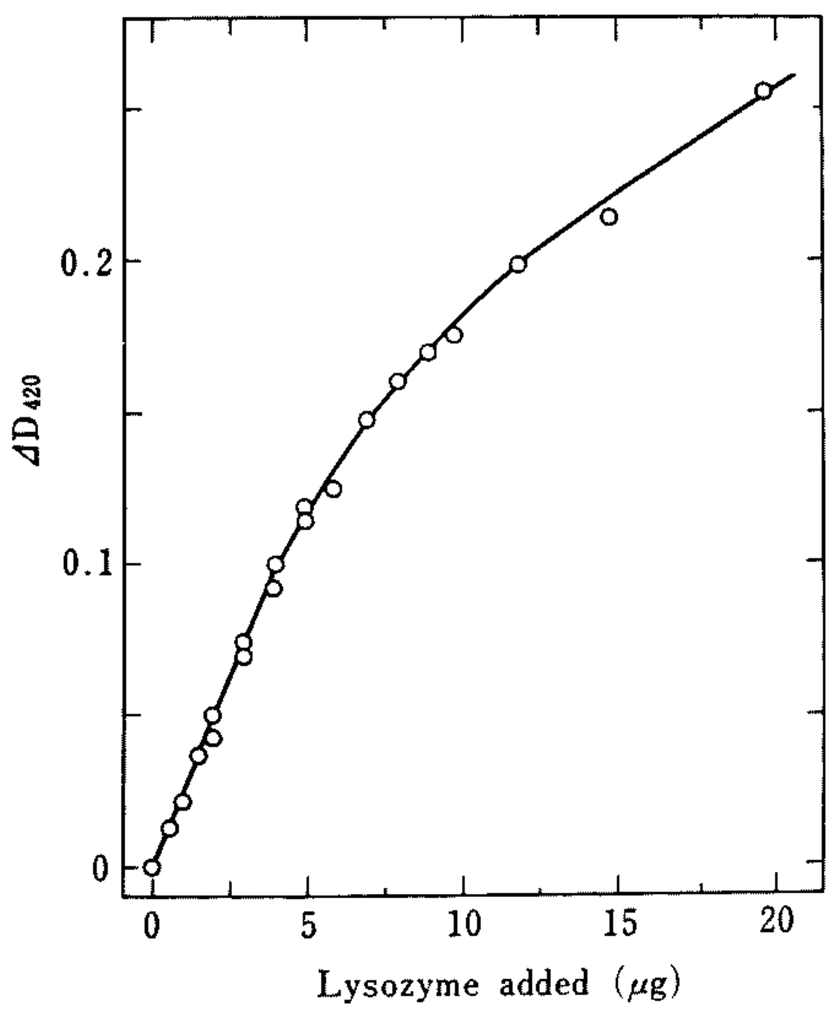

FIG. 2. Standard Curve for the Lysozyme Activity in 0.1 M Acetate Buffer, pH 4.5.

A standard curve for lysozyme concentration is shown in Fig. 2. A correction was applied for decolorization caused by lysozyme itself at each enzyme concentration. Decrease in O.D.$_{420}$(ΔD_{420}) was 3.0/mg lysozyme. If lysozyme concentration is 10 μg, the correction is 0.030 (ΔD_{420}).

As a test experiment, the activities of NBS-

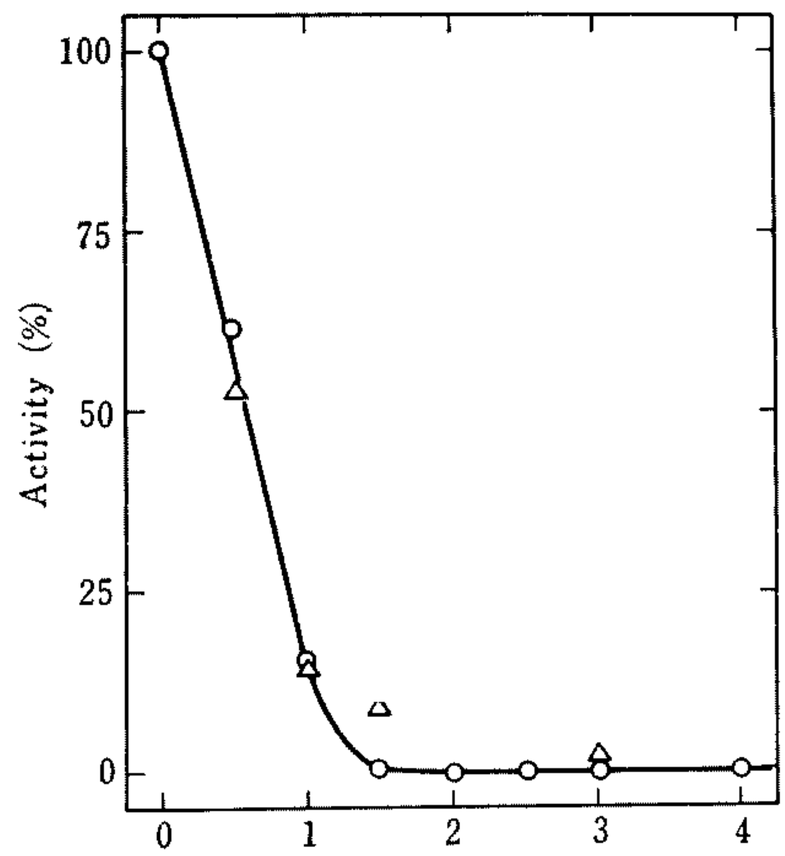

FIG. 3. Activity of NBS-Oxidized Lysozyme.

NBS oxidation was performed in 0.1 M acetate buffer, pH 4.5. The activity was measured under the same conditions as in Fig. 2 using 10 μg enzyme (○). The activity measured with viscosimetry was noted for comparison (△).

7) J. T. Park and M. J. Johnson, *J. Biol. Chem.*, **181**, 149 (1949).
8) M. Somogyi, *J. Biol. Chem.*, **195**, 19 (1952).
9) R. Senzyu and S. Okimasu, *Nippon Nogeikagaku Kaishi*, **23**, 432 (1950).

oxidized lysozyme[10] were followed using the standard curve obtained above and is shown in Fig. 3. The lysozyme concentration was kept at 10 μg. Note the good agreement with activity determined using viscosimetry.

Thus a simple and very reproducible activity measurement for lysozyme has been developed. By following the initial rate of the reaction, more accurate kinetic data for lysozyme may be obtained and this is currently under investigation.

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