DEAE-Dextran Enhanced Firefly Bioluminescent Assay of ATP

Tamio Kamidate,* Toshikazu Kinkou, and Hiroto Watanabe
Faculty of Engineering, Hokkaido University, Sapporo 060

(Received November 20, 1995)

Diethylaminoethyl (DEAE) - Dextran (Dx) enhanced the intensity of bioluminescence from firefly luciferase-luciferin reaction with adenosine-5'-triphosphate (ATP). The detection limit for ATP was 5 x 10^{-12} M in the presence of DEAE-Dx, and was improved a factor of three times compared to that in the absence of DEAE-Dx.

Firefly luciferase catalyses the oxidative decarboxylation of luciferin in the presence of ATP and Mg^{2+} to yield light emission. The amount of light can be related to the amount of ATP. The firefly bioluminescent (BL) assay of ATP has been applied to the evaluation of the microbial pollution, because ATP is present in all living micro-organisms. Recently, surfactant micelles and cycrodextrins (CD) have been successful in improving the sensitivity of chemiluminescence (CL) method.[1] On the other hand, some organic solvents and surfactants are known to affect the reaction rate and catalytic activity of the firefly luciferase-luciferin reaction.[2,3] However, no reports have been found on the application of micelles and polyelectrolytes such as CDs and polysaccharides to the improvement of sensitivity of the firefly bioluminescent (BL) assay of ATP.

In the course of our studies on enhancing the BL intensity using polysaccharides, we have found that DEAE-Dx gave the BL enhancement effect. Based on this finding, a highly sensitive BL assay of ATP has been developed.

Firefly luciferase and D-luciferin were purchased from Sigma Chemical Co. Dx T-500, Dx-Sulfate and DEAE-Dx were bought from Pharmacia Biotech. Chitosan and λ-carrageenan were purchased from Fulka BioChemika and Wako Chemicals Co.

The general BL experimental procedure consisted in pipetting 100 μl of a BL reagent solution and 50 μl of polysaccharides or micelles solution into a cuvette in a luminometer. The BL reagent solution was prepared with 25 mM (1 M = 1 mol dm^{-3}) HEPES buffer (pH 7.8). The BL reagent contained 50 nM luciferase, 150 μM luciferin, 6.0 mM Mg^{2+}, 0.5 mM dithiothreitol (DTT), 2.0 mM EDTA and 1.5 mg l^{-1} bovine serum albumin (BSA). Next, a 250 μl portion of ATP solution was injected into the cuvette. Thus the BL reaction was initiated and the light output was counted with a CL detector (TD-3A;Tohoku Denshi Sangyo Co.,Ltd.). The resultant photoncurrent was converted to a voltage and displayed on a chart recorder.

The BL intensity was defined as a maximum photon counts. The relative BL intensity was referred to as the ratio of the BL intensity counted in the presence of micelles or polysaccharides to that in the absence of micelles or polysaccharides. The relative BL intensity shown in fugures were indications of the mean of three successive measurements. The detection limit for ATP was defined as the concentration of ATP that produced the BL intensity equal to five the blank intensity counted in the mixture containing no ATP.

In order to investigate whether a medium of surfactant agent can function effectively for enhancing the BL intensity, different charge-type of surfactant micelles were added to the BL reagent solution. Three surfactants, one of each of the three main types of surfactants, were studied: sodium dodecylsulfate (SDS, an anionic surfactant), cetyltrimethylammonium bromide (CTAB, a cationic surfactant) and Triton X-100 (a nonionic surfactant). The critical micelle concentration for these surfactants were 0.2 - 0.4 mM for Triton X-100, 9.8 mM for SDS and 0.4 - 0.9 mM for CTAB, respectively.[4] Figure 1 shows relative BL intensiy-surfactant concentration profiles for the reaction with 4.0 nM ATP. The surfactnat concentrations shown in Figure 1 are the final values in the reaction mixture. The BL intensity decreased with increasing surfactant concentration, regardless of the type of surfactant employed. These results indicate that micellar media are not effective as a reaction medium for enhancing the BL intensity.

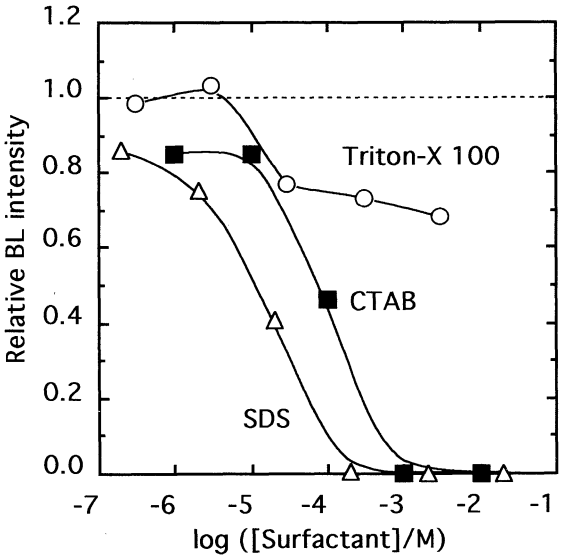

Figure 1. Effect of surfactant concentration on relative BL intensity.

The influence of β-CD concentration on the BL intensity was examined in the range 0.001 - 1.0 wt%. When β-CD concentration was below 0.1 wt%, the relative BL intensities were about 1.0. However, the relative BL intensity rapidly decreased above 0.1 wt% β-CD.

We carried out the BL measurements with 4.0 nM ATP in the presence of different charge-type of polysaccharides: Dx T-500 (a nonionic type), DEAE-Dx and Chitosan (a cationic type), and Dx-Sulfate and λ-carrageenan (an anionic type). Typical BL response curves are shown in Figure 2. In the case of any polysaccharides, the light emission appeared rapidly from the start of the reaction, and reached its maximum intensity after which the intensity of the light emission decreased gradually. The presence of such a cationic polysaccharide as DEAE-Dx and Chitosan enhanced the BL intensity when compared with that water alone. When Dx T-500 was used, no BL enhancement was observed. In constrast, the BL intensity decreased rapidly in the presence of such an anionic polysaccharides as Dx-Sulfate and λ-carrageenan.

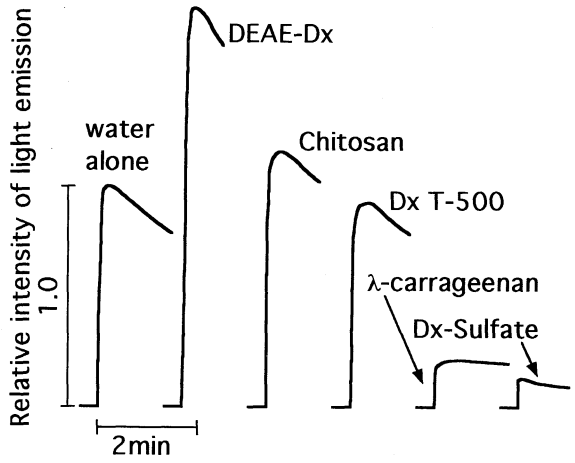

Figure 2. Typical BL response curves in the presence of polysaccharides.

DEAE-Dx: 2.0wt%, Chitosan: 0.02wt%, Dx T-500: 2.0wt%, λ-carrageenan: 0.1wt%, Dx-Sulfate: 0.002wt%.

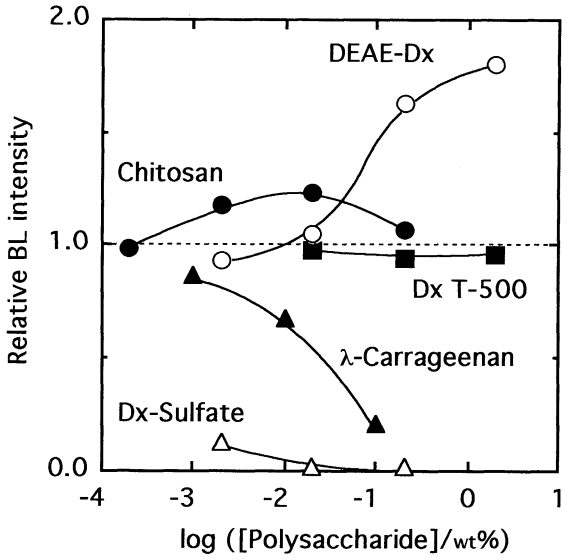

Figure 3. Effect of polysaccharide concentration on relative BL intensity.

The effect of polysaccharide concentration on the relative BL intensity were investigated. Figure 3 shows relative BL intensity-polysaccharide concentration profiles for the reaction with 4.0 nM ATP. The polysaccharide concentrations shown in the figure are the initial values in the solution prepared. The effect of Dx species concentration on the BL intensity were investigated in the range 0.002 - 2.0 wt%, since the Dx solutions were very viscous above 2.0 wt%. The relative BL intensity increased with an increase in DEAE-Dx concentration, and exhibited a maximum at 2.0 wt% DEAE-Dx. A broad maximum of the relative BL intensity was observed at 0.02 wt% Chitosan. The relative BL intensity was independent on Dx T-500 concentrations added. In contrast, the relative BL intensity decreased with increasing Dx-Sulfate and λ-carrageenan concentrations.

As can be seen in Figure 2, the enhancement of the BL intensity was dependent on the charge of polysaccharides used. The cationic polysaccharides such as DEAE-Dx is more effective than other charge types of polysaccharides in enhancing the BL intensity. Therefore, the BL enhancement in the presence of DEAE-Dx could be explained in terms of electrostatic interaction between BL reactants and DEAE-Dx. That is, DEAE-Dx is present in a positive form in the reaction condition(pH=7.8). On the other hand, the deprotonated forms of luciferin and ATP are expected to be the main species in the reaction mixture. Consequently, the effective local concentrations of luciferin and ATP at DEAE group of Dx is greater than their stoichiometric concentration in bulk water alone. Thus, the rate of the BL reaction could be greater in the solution containing DEAE-Dx than in water alone, resulting the increase of the BL intensity in the preseence of DEAE-Dx. However, the exact function of DEAE-Dx upon the luciferase-luciferin reaction are still not clear.

In subsequent studies, the optimum conditions for the quantification of ATP were determined by measuring the BL intensities, so as to be maximal under optimum conditions. The influence of luciferin concentration was tested in the range 1.0 - 250 μM. The BL intensity increased with increasing luciferin concentration up to 150 μM, after which it leveled off with increasing luciferin concentration. Thus, the optimum luciferin concentration was determined to be 150μM. The effect of Mg^{2+} concentration was examined in the range 2 - 14 mM. The BL intensity exhibited a broad maximum at 6 mM Mg^{2+}. The optimum Mg^{2+} concentration was thus chosen to be 6 mM. The optimum concentrations of EDTA, DTT and BSA were determined by taking into account their effects on luciferase activity during long term storage.

Analytical calibration curve was prepared under the optimized experimental conditions. Logarithmic calibration curve of ATP in the presence of DEAE-Dx and in the absence of DEAE-Dx were linear over ranging in initial concentration from the detection limit of 2.5×10^{-12} M and 8.3×10^{-11} M up to 2.5×10^{-9} M with slope of 0.85, respectively. Therefore, the detection limit in the presence of DEAE-Dx is improved by factors of three compared with that in water alone. The relative standard deviation of five succesive experiments in DEAE-Dx was 5.6% at 2.5×10^{-10} M of ATP.

In conclusion, the best sensitivity of the BL reaction of ATP is achieved by DEAE-Dx. Further studies on the mechanism of the BL enhancement in DEAE-Dx are under way.

References and Notes

1 T.E.Reihl, C.L.Malehorn, and W.L.Hinze, *Analyst*, **111**, 931 (1986).
2 L.J.Kricka and M.DeLuca, *Arch.Biochem.Biophys.*, **217**, 674 (1982).
3 W.J.Simpson and J.R.M.Hammond, *J.Biolumin.Chemilumin.*, **6**, 97 (1991).
4 I.M.Warner and L.B.Mcgown, "Advances in Mulitidimensional Luminescence," JAI Press Inc., London (1991).