shows *Pvu*II (lane 1) or *Not*I (lane 2) fragments of genomic *B. bronchiseptica* DNA that cross-hybridize with the 2.9-kb fragment of the *B. pertussis fha* gene. A doublet in lane 1 is obscured because of background exposure of the X-ray film resulting from discharge of static electricity as the film was removed from the surface of the pouch containing the membrane. Subsequent exposures resulted in similar background that was visible on other areas of the film. A film devoid of background can usually be obtained if a sufficient number of additional exposures are attempted. However, since the film costs roughly $2.00 (USA) per sheet, this can be an expensive solution. Figure 1B is an identical exposure using the same membrane as in Figure 1A. However, before removing the film to be developed from the surface of the plastic pouch, the antistatic instrument was discharged by holding it within 12 in. of the film’s surface and slowly squeezing and releasing the trigger over a period of about 5 s. The pinpoint background seen in Figure 1A was completely eliminated by this step.

The antistatic device is also useful for eliminating background exposure from film used for other membrane-based procedures, including northern blots and colony lifts. Avoidance of such background is especially desirable when working with colony lifts, since the small, circular signals can be mistaken for colonies to which the probe of interest has hybridized. In some cases, careful alignment of the membrane with the plate used for the colony lift reveals that the signals do not line up with colonies. However, it can be difficult to accurately align the membrane with the plate, especially if semi-confluent or confluent growth is present. Furthermore, the device is helpful when working with small strips or pieces of nitrocellulose for blotting techniques. Such small pieces are often extremely difficult to handle and cling tenaciously to forceps and plastic surfaces because of static electricity. The antistatic device easily and rapidly abolishes this problem.

In summary, the use of an antistatic device eliminates problematic background exposure from X-ray film. Although used here in conjunction with a chemiluminescent detection procedure, those using 32P might also find use of the antistatic device to be helpful since exposure of film to static discharge from clothing or hair is a common problem. The cost of the device is quickly recovered when one considers the cost of film that is otherwise discarded because of unacceptable background.

**REFERENCE**


*CSPD is a register trademark of Tropix, Bedford, MA, USA. Product names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the United States Department of Agriculture (USDA) implies no approval of the product to the exclusion of others that may also be suitable. Address correspondence to Dr. Karen Register, Swine Respiratory Diseases Project, USDA/ARS/National Animal Disease Center, P.O. Box 70, 2300 Dayton Road, Ames, IA 50010, USA. Internet: kregiste@nadc.ars.usda.gov*

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**Enhanced Chemiluminescent Assay for Transglutaminases**

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Chemiluminescence (CL) is a sensitive technique for enzyme assays (3–5). *N*-aminobutyl-*N*-ethylisoluminol (ABE-I) was previously shown to be a CL substrate for blood coagulation factor XIIIa (FXIIIa) and tissue transglut-
aminase (Tg) (1). ABE-I, covalently conjugated to \(N,N'-\text{dimethylcasein by Tg or FXIIIa catalysis, emitted CL following oxidation (1). Recently it was shown that under suitable oxidizing conditions, L-carnosine (\(N\beta\text{-alanyl}-L\text{-histidine}) was a potent enhancer of free (non-conjugated) ABE-I CL (2). Enhancement of CL by L-carnosine was especially strong when the combination of 20 mM \(K_2Fe(CN)_6\) and 10 mM \(H_2O_2\) was the oxidizer (2). The present work was undertaken with three objectives: (i) to determine whether L-carnosine was also an enhancer of covalently conjugated ABE-I CL, (ii) to compare the light emissions from protein-ABE-I conjugate oxidized using previously reported \(NH_2S_2O_8\) (1) vs. the recently discovered combination of \(K_2Fe(CN)_6\) \(H_2O_2\), and L-carnosine (2) and (iii) to develop an enhanced CL assay for Tg and FXIIIa.

Partially purified Tg (Sigma, St. Louis, MO, USA) activity was quantitated by measuring the CL from ABE-I (Sigma) that was covalently conjugated to \(N,N'-\text{dimethylcasein (Calbiochem-Novabiochem, La Jolla, CA, USA) by Tg catalysis as described previously (1). Purified recombinant human FXIII (a gift from Dr. Paul Bishop, Zymogenetics, Seattle, WA, USA) was activated using human \(\alpha\)-thrombin (Haematologic Technologies, Essex Junction, VT, USA) as described previously (1). Activated FXIII (FXIIIa) catalysis was quantitated by measuring the covalent conjugation of ABE-I into \(N,N'-\text{dimethylcasein (1). The protein-amine conjugate was separated from free ABE-I using trichloroacetic acid precipitation and washing (1). The washed protein-amine precipitate was dissolved in 100 mM NaOH, and portions of the solution were oxidized using either 15 mM \((NH)_2S_2O_8\) (Sigma) in \(H_2O\) or 20 mM \(K_2Fe(CN)_6\) (Sigma) plus 10 mM \(H_2O_2\) (Fisher Scientific, Pittsburgh, PA, USA) in 100 mM NaOH as described previously (1,2). The protein-amine conjugate was oxidized by \(K_2Fe(CN)_6\) \(+ H_2O_2 \pm 5 \text{ mM L-carnosine (Bachem, King of Prussia, PA, USA). L-Carnosine was added to the protein-amine conjugate solution before oxidation. CL from the oxidized protein-amine conjugate was measured using an Optocomp® II Luminometer (MGM Instruments, Hamden, CT, USA) (1,2). CL for the total sample volume was then calculated, and the data are presented as the mean of triplicate measurements ± standard deviation. Affinity constants were calculated using EnzymeKinetics Version 1.2 software (Trinity Software, Campton, NH, USA) (1) installed on a Macintosh® SE computer (Apple Computer, Cupertino, CA, USA).

L-Carnosine was indeed a potent CL enhancer of not only free ABE-I (2) but also of covalently conjugated ABE-I (Figure 1). Although the signal/noise (S/N) ratio was highest at 1 mM L-carnosine, all further experiments involving L-carnosine were conducted using a 5-mM concentration of the dipeptide. The higher concentration was chosen because of the nearly 7-fold increase in light emission at this concentration vs. at 1 mM L-carnosine (Figure 1). The S/N at 5 mM L-carnosine was 81, compared with 119 at 1 mM (Figure 1). This reduction in S/N at 5 mM L-carnosine was considered acceptable in view of the greater sensitivity obtained. Although CL output appeared to increase linearly beyond 10 mM L-carnosine (Figure 1), higher concentrations were not tested because of the decline in S/N. The greater sensitivity also allowed a reduction of light emission count time from 30 (1) to 10 s (present work).

CL from the protein-amine conjugate was compared next using the two oxidizer systems. There was a linear \((r^2 = 0.990)\) increase of light emission from the \(N,N'-\text{dimethylcasein-ABE-I covalent conjugate with time of Tg catalysis following oxidation by } K_2Fe(CN)_6 \pm H_2O_2\) in the presence of L-carnosine (Figure 2). At 60 min of catalysis time, a >50-fold increase in CL was obtained from the protein-amine conjugate oxidized by \(K_2Fe(CN)_6 \pm H_2O_2\) in the presence of L-carnosine vs. light emission obtained using \((NH)_2S_2O_8\) as the oxidizing agent (Figure 2). For FXIIIa catalysis under these conditions, a 22-fold increase was observed, and the reaction was fairly linear between 10–60 min \((r^2 = 0.958)\) (Figure 2). Under appropriate conditions, Tg or FXIIIa reaction times could be decreased to just 5 min. To illustrate, at 5 min, the CL from Tg-catalyzed protein-amine conjugate oxidized using either \((NH)_2S_2O_8\) or \(K_2Fe(CN)_6 \pm H_2O_2\) + 5 mM L-carnosine were 9040 ± 2736 and 237 600 ± 82 400 relative light units (RLU), respectively (Figure 2). These results represent a nearly 30-fold higher light emission from the protein-amine covalent conjugate using the new oxidizer plus enhancer combina-

![Figure 1. Establishing the optimum L-carnosine concentration. ABE-I (2.5 mM) was covalently conjugated to \(N,N'-\text{dimethylcasein (1 mg/mL) using 10 µg/mL Tg at 37°C for 60 min (1). The protein-amine conjugate was oxidized with 20 mM \(K_2Fe(CN)_6 \pm 10 \text{ mM } H_2O_2\), with the sample containing the indicated concentrations of L-carnosine. Closed circles indicate RLU, and open circles indicate S/N.](image)
Benchmarks

The corresponding values for FXIIIa catalysis at 10 min were, respectively, $63.960 \pm 11.560$ and $1791433 \pm 349464$ RLU (Figure 2), yielding a 28-fold higher light emission in the presence of L-carnosine. The kinetic parameters ($K_m$) for Tg-catalyzed ABE-I conjugation to $N,N'$-dimethylcasein using the new oxidizer-enhancer combination was $0.467 \pm 0.101$ mM. This value was close to the $K_m$ ($0.399 \pm 0.087$ mM) determined previously using $(NH)_4S_2O_8$ (1).

The data confirm, as expected, that while the sensitivity of the Tg-CL assay was enhanced with the new oxidizer plus enhancer combination, the kinetic parameters remained similar.

Light emission from the protein-amine conjugate catalyzed by increasing amounts of Tg or FXIIIa was compared next. Light emission from the Tg-catalyzed, protein-amine conjugate was linear ($r^2 = 0.980–0.995$) over the range of Tg concentrations tested, using the two different oxidizer systems both alone and in the presence of L-carnosine (Figure 3). However, total light emission varied considerably depending upon both the choice of oxidizer and presence of L-carnosine. For example, with 10 $\mu$g/mL Tg RLUs from the protein-amine conjugate oxidized using either (i) $(NH)_4S_2O_8$, (ii) $K_3Fe(CN)_6 + H_2O_2$ or (iii) $K_3Fe(CN)_6 + H_2O_2 + 5$ mM L-carnosine were $45668 \pm 6130$, $21205 \pm 1150$ and $1035244 \pm 244578$, respectively (Figure 3). These numbers reflected a 23-fold higher CL from the Tg-catalyzed, protein-amine conjugate oxidized using the new oxidizer and enhancer vs. $(NH)_4S_2O_8$. Similar results were obtained from FXIIIa-catalyzed $N,N'$-dimethylcasein-ABE-I covalent conjugate as well (Figure 3). For example, with 5 $\mu$g/mL FXIIIa, RLUs from the protein-amine conjugate oxidized using (i) $(NH)_4S_2O_8$, (ii) $K_3Fe(CN)_6 + H_2O_2$, or (iii) $K_3Fe(CN)_6 + H_2O_2 + 5$ mM L-carnosine were $774123 \pm 91737$, $449820 \pm 30396$ and $26948300 \pm 2413480$, respectively (Figure 3). These values correspond to a 35-fold higher CL from the FXIIIa-catalyzed, protein-amine conjugate oxidized using the new oxidizer plus enhancer combination vs. $(NH)_4S_2O_8$.

Light emission increased linearly ($r^2 = 1.000$) from $N,N'$-dimethylcasein-ABE-I covalent conjugate catalyzed by 0.5–5.0 $\mu$g/mL of FXIIIa.

Tg activity was also measured in the presence and absence of the competitive amine inhibitor ImmunoPure® 5 (Biotinamido)pentylamine (Pierce Chemical, Rockford, IL, USA). At 0.5 mM, this compound produced 52% ± 4% inhibition of 2.5 mM ABE-I conjugation to 2 mg/mL $N,N'$-dimethylcasein catalyzed by 100 $\mu$g/mL Tg during 60 min at 37°C. However, light emission from the inhibited protein-amine covalent conju-
These results demonstrated once again that the sensitivity of Tg detection was greatly improved using the new oxidizer plus enhancer combination. Lower amounts of the enzyme could be determined during shorter catalysis durations, and Tg activity could be measured with improved sensitivity even in the presence of inhibitors.

The mechanism whereby L-carnosine enhances CL of free, non-conjugated aminophthalhydrazides (2) or \(N,N'-\)dimethylcasein-ABE-I covalent conjugate (present work) is not entirely clear. It is possible that L-carnosine might prolong the half-life \(t_{1/2}\) of superoxide anion by forming L-carnosine-radical adducts, thus facilitating intermolecular energy transfer (2). L-Carnosine did not provide similar magnitudes of enhancement of CL from aminophthalhydrazides when \((\text{NH}_4)_2\text{S}_2\text{O}_8\) was used as the oxidizer (2). In conclusion, the enhanced CL assay could be used to monitor FXIIIa levels in patients with bleeding disorders that are caused by congenital or acquired FXIIIa inhibitors. The enhanced CL assay could also facilitate the development of film-format assays for FXIIIa and/or Tg.

**REFERENCES**


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Centrifugation is a widely used and important separation technique in biological research. To set up the centrifugation conditions as desired, it is important to accurately convert the relative centrifugal force (RCF) from speed and vice versa. In addition, equivalent centrifugation conditions often need to be determined when procedures described in the literature are adapted to the centrifuge and rotor types available in the laboratory. There are three conventional methods to perform the conversion between the RCF and rotor speed: 

(i) to use the nomograms such as those provided in a laboratory manual (1); 

(ii) to use the RCF and rotor speed conversion tables provided by the manufacturer to find the closest match to the desired RCF and rotor speed values; and 

(iii) to use the following formula, which requires prior knowledge of rotor specifications, such as the radius (R) in millimeters, to directly calculate the RCF (Equation 1) and rotor speed (Equation 2).

$$\text{RCF}(g) = 1.117 \times R \times \frac{\text{RPM}(1000)^2}{\text{rpm}}$$ \hspace{1cm} [Eq. 1]

$$\text{RPM}(\text{rpm}) = 1000 \times \frac{\text{RCF}(1.117 \times R)^{1/2}}{}$$ \hspace{1cm} [Eq. 2]

On the other hand, K factors, which determine the necessary sedimentation time, are needed to calculate the equivalent run time of one rotor as compared with the other. As shown in Equation 3, the K factor is determined by rotor speed and the ratio of the maximum and minimum radius.

$$K = \ln \left( \frac{R_{\text{max}}}{R_{\text{min}}} \right) / \text{RPM}^2 \times \frac{2.533 \times 10^{11}}{}$$ \hspace{1cm} [Eq. 3]

Because K factors listed in rotor manuals are usually calculated using the maximum speed of the rotor, it is necessary to recalculate the K factor according to the actual speed.

As most laboratories have internet access, researchers find the World Wide Web (WWW) a rich source for biological information and research tools. Utility programs written in JavaScript have previously been described as useful tools for biological research (2,3). Here, we describe a JavaScript-based computer program, named CentrifugeHelper, as a convenient method to access a collection of about four hundred commonly used rotor types, calculate the RCF and RPM values and determine equivalent running conditions between two rotors.

CentrifugeHelper uses a graphical user interface (GUI) in a table format (Figure 1). In the first section, it allows users to select the specific rotor from the pulldown menus, which alphabetically list rotor types from Sorvall, Beckman Instruments, Composite, IEC, Eppendorf and Piramoon. Upon the selection, the program displays rotor specifications, including the maximum, average and minimum radius, maximum speed, the K factor at the maximum speed, maximum, average and minimum RCF at the maximum speed, number of tubes and volume of each tube for the selected rotor and the total rotor capacity. In addition, a button entitled Enter parameters manually is also provided to let users enter the rotor specifications to perform a calculation on a custom rotor. In the next section, users can calculate the RCF and the K factor by entering the desired speed in rpms and clicking on the Calculate button. Similarly, users can calculate the rotor speed and the K factor by entering the desired RCF and clicking on the Calculate button. In these calculations, the program compares the input RCF and rotor speed with the