Development of a microtitre plate-based assay for lipid-linked glycosyltransferase products using the mycobacterial cell wall rhamnosyltransferase WbbL

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In *Mycobacterium tuberculosis* a rhamnosyltransferase (WbbL) catalyses the transfer of an α -L-Rhap residue from dTDP-L-rhamnose (dTDP-Rha) to decaprenyldiphosphoryl- α -D-N-acetylglucosamine (GlcNAc-P-P-DP) to form α -L-Rhap-(1 $\rightarrow$ 3)- α -D-GlcNAc-P-P-DP, which is then further elongated with Gal and Ara units, and finally mycolylated and attached to the peptidoglycan. This enzyme is essential for *M. tuberculosis* viability and at the same time absent in eukaryotic cells, and is therefore a good target for the development of new antituberculosis therapeutics. Here, we report a microtitre plate-based method for the assay of this enzyme using a crude membrane preparation from an *Escherichia coli* strain overexpressing *wbbL* as an enzyme source and the natural acceptor substrate GlcNAc-P-P-DP. Initial characterization of the enzyme included unequivocal identification of the product Rha-GlcNAc-P-P-DP by liquid chromatography (LC)-MS, and the facts that WbbL shows an absolute requirement for divalent cations and that its activity is stimulated by β -mercaptoethanol. Its pH optimum and basic kinetic parameters were also determined, and the kinetic analysis showed that WbbL uses a ternary complex mechanism. The microtitre plate-based assay for this enzyme was developed by taking advantage of the lipophilic nature of the product. This assay should be readily transferable to other glycosyltransferases which use lipid-based acceptors and aid greatly in obtaining inhibitors of such glycosyltransferases for new drug development.

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INTRODUCTION

Many bacterial glycosyltransferases (GTs) involved in cell wall biosynthesis transfer a sugar from a sugar nucleotide to a lipid (often polyisoprene)-containing acceptor. The N-acetylglucosamine (GlcNAc) transferase MurG is a key peptidoglycan synthetic enzyme that utilizes a lipid-containing acceptor (Mengin-Lecreux *et al.*, 1991). Another important example in Gram-positive bacteria is TagA (Zhang *et al.*, 2006), by which an N-acetylmannosamine is attached to an undecaprenyl diphosphate-linked GlcNAc. In the mycobacterial cell wall the biosynthesis of the polymer arabinogalactan, which connects the peptidoglycan and mycolic acid layers, occurs on the lipid decaprenyl phosphate (DP) (Mikusova *et al.*, 1996). Here, a rhamnosyl residue is attached to a decaprenyl diphosphate-linked GlcNAc by the rhamnosyltransferase WbbL (Mills *et al.*, 2004). Convenient and inexpensive assays to search for

inhibitors of such enzymes are lacking, and using WbbL we describe herein the development of such an assay.

The *wbbL* gene is found in the genome of all mycobacteria. The protein sequence of this enzyme does not show homology to other rhamnosyltransferases in mycobacteria that synthesize various glycolipids. Previous studies with the temperature-sensitive mutant of *Mycobacterium smegmatis* mc²155 (Mills *et al.*, 2004) have shown that WbbL is essential for mycobacterial viability. The incubation of the temperature-sensitive mutant at the non-permissive temperature results in bacteria which cannot be recovered at the lower permissive temperature, suggesting that the inactivation of WbbL is a lethal defect (Mills *et al.*, 2004). Therefore, a facile assay for its activity is particularly important in the development of new tuberculosis (TB) drugs.

The substrates for WbbL are dTDP-L-rhamnose (dTDP-Rha) and decaprenyldiphosphoryl-D-N-acetylglucosamine (GlcNAc-P-P-DP) (Fig. 1). GlcNAc-P-P-DP has hitherto not been available, and all assays for WbbL have been done using crude membranes that contain the GlcNAc-1-P transferase WecA and endogenous DP, which allows the *in*

Abbreviations: DP, decaprenyl phosphate; dTDP-Rha, dTDP-L-rhamnose; ESI, electrospray ionization; GlcNAc-P-P-DP, decaprenyldiphosphoryl- α -D-N-acetylglucosamine; GT, glycosyltransferase; LC, liquid chromatography; TB, tuberculosis.

Fig. 1. The reaction catalysed by rhamnosyltransferase (WbbL).

situ preparation of the GlcNAc-P-P-DP acceptor when UDP-GlcNAc is provided. Although such assays have been used to identify the gene encoding WbbL (Mills *et al.*, 2004), they are clearly inadequate to characterize the enzyme and to develop specific assays for its activity. Using WbbL substrates directly, we describe for what is believed to be the first time the characterization of WbbL in terms of pH optimum, ion dependence and kinetics, and then go on to describe the development of a microtitre plate-based assay for its activity that takes advantage of the lipid nature of the enzyme product.

METHODS

PCR, cloning, and expression. The DNA sequence of the *Mycobacterium tuberculosis* wbbL gene (906 bp) was acquired from the *M. tuberculosis* genome database (<http://genolist.pasteur.fr/TubercuList/>). *M. tuberculosis* wbbL was amplified from *M. tuberculosis* H37Rv genomic DNA by Vent DNA polymerase (New England Biolabs) using CATATGGTAGCGGTGACCTAC and GGATCCTCAGTGGCCGCCCTTC primers. NdeI and BamHI sites in the primers facilitated cloning into plasmid pSTBlue 1. The resulting pSTB-Mtb wbbL plasmid was digested by NdeI and BamHI, and the wbbL gene was ligated into the NdeI and BamHI sites of plasmid pET16b, yielding the expression vector pET16b-Mtb wbbL. For expression, pET16b-Mtb wbbL was transformed into the following *Escherichia coli* strains: BL21[DE3] (Novagen), BL21[DE3]pLysS (Novagen), BL21[DE3] codon plus (Novagen), C41DE3 (Miroux & Walker, 1996), Arctic Express (Stratagene), ER2566 (New England Biolabs), and BL21[DE3] containing pKJE7 (Takara Mirus Bio). Expression from pET16b in *E. coli* (ER2566) produced substantial WbbL in the membrane fraction and was used in further studies.

Cells (*E. coli* ER2566) were grown at 37 °C in LB medium containing 100 µg ampicillin ml⁻¹ until the culture reached OD₆₀₀ 0.6, and were then induced with 0.5 mM IPTG for 4 h at room temperature. The cells were harvested, then resuspended in 100 mM TAPS buffer, pH 8.6, containing 5 mM β-mercaptoethanol and 10 mM MgCl₂ (buffer 1) and broken by French press. The broken cells were centrifuged at 20 000 g

for 10 min and the obtained supernatant was ultracentrifuged at 100 000 g for 2 h. The pellet containing the membrane fraction was homogenized in buffer 1 and stored at -80 °C.

SDS-PAGE. SDS-PAGE was run using 12 % gels, and proteins were visualized with Coomassie brilliant blue and analysed by Western blotting using monoclonal anti-polyhistidine antibody (mouse IgG2a isotype; Sigma) as the first antibody and anti-mouse-IgG-alkaline phosphatase conjugate antibody (Sigma) as the second antibody.

dTDP-Rha and dTDP-[¹⁴C]Rha preparation. dTDP-Rha was made from dTDP-Glc (Sigma-Aldrich) using recombinant RmlB (conversion to dTDP-4-keto-6-deoxy-D-xylohexulose), RmlC (di-epimerization to form dTDP-4-keto-6-deoxyl-L-lyxo-hexulose) and RmlD (reduces the 4-keto group to form dTDP-Rha) (Mills *et al.*, 2004). The standard 1 ml reaction mixture contained 1.2 mM dTDP-Glc, 1.8 mM NADPH, 20 mM MgCl₂, 53 µg RmlB from *Salmonella typhimurium*, 13 µg RmlC (*M. tuberculosis*) and 5 µg RmlD (*M. tuberculosis*) in 200 mM Tris/HCl, pH 7.5. After 1 h incubation at 30 °C, the reaction mixture was centrifuged using an Amicon Microcon with 10 kDa cut-off membranes to remove proteins. dTDP-Rha was purified by HPLC on a Dionex column (CarboPac, PA-100). The HPLC flow rate was 2 ml min⁻¹ and a gradient from 0 to 500 mM triethylammonium acetate over 30 min was applied. The fractions were monitored by reading A₂₆₀ and checked by analytical HPLC (isocratic gradient of 200 mM KH₂PO₄). The retention times for dTDP-Rha and dTDP-Glc were 5.74 and 6.52, respectively. The fractions containing dTDP-Rha were pooled and lyophilized. For quantification of dTDP-Rha, alditol acetates were prepared and analysed by GC-MS. dTDP-[¹⁴C]Rha was made as described elsewhere (Mills *et al.*, 2004). Briefly, [¹⁴C]sucrose (American Radiolabelled Chemicals) was converted to [¹⁴C]Glc-1-P by commercially available sucrose phosphorylase. Subsequently, [¹⁴C]Glc-1-P and TTP were converted to dTDP-[¹⁴C]Glc by RmlA. The conversion of dTDP-[¹⁴C]Glc to dTDP-[¹⁴C]Rha and subsequent purification were performed as described above for non-radioactive dTDP-Rha.

Enzymic synthesis and purification of GlcNAc-P-P-DP. Enzymic synthesis of GlcNAc-P-P-DP was adopted from Rush *et al.* (1997). GlcNAc-P-P-DP was synthesized from DP (Indofine) and UDP-GlcNAc using WecA enzyme. The membranes overexpressing WecA enzyme were prepared as described elsewhere (Hyland & Anderson, 2003). The reaction mixture contained 0.5 mg membrane protein, 50 mM Tris, pH 8.0, 5 mM β-mercaptoethanol, 40 mM MgCl₂, 0.5 % CHAPS, 1 mM sodium orthovanadate, 4 mM UDP-GlcNAc and 2 mM DP in a total volume of 0.5 ml. After incubation for 1.5 h at 30 °C, the reaction was quenched by the addition of 7 ml chloroform:methanol (2:1, v/v) and shaken at room temperature for 20 min. After addition of 500 µl water, the organic layer was dried. The organic fraction was dissolved in 0.2 ml 0.2 M NaOH in CH₃OH and incubated for 20 min at 37 °C (Mikusova *et al.*, 1996). After neutralization of NaOH with 5 µl CH₃COOH, the reaction mixture was dried and resuspended in 1.75 ml chloroform:methanol:water (4:2:1, by vol.). After extraction, the organic layer was dried and applied to an aluminium oxide column (0.5 × 5 cm). The column was developed with six column volumes of chloroform:methanol:ammonium hydroxide:water (6.5:2.5:0.2:0.2, by vol.), followed by eight volumes of chloroform:methanol:ammonium hydroxide:water (10:10:0.046:3, by vol.). The fractions were checked by TLC (chloroform:methanol:ammonium hydroxide:water; 65:25:0.5:3.5, by vol.). The fractions containing GlcNAc-P-P-DP were pooled and dried. The yield, after quantification of GlcNAc-P-P-DP by preparation of alditol acetates followed by GC-MS analysis, was 0.25 mg, providing enough substrate for five microtitre plates using the microtitre plate assay described below.

Deprotection of per-*O*-acetylated GlcNAc-P-P-DP. per-*O*-Acetylated GlcNAc-P-P-DP was provided as a gift by Drs Michio Kurosu and Kai Li and prepared by them as described by Kurosu & Li (2008), using acetylated GlcNAc-1-P (Imperiali & Zimmerman, 1990) in the coupling reaction with DP. The *O*-acetylated product was purified by preparative TLC (RP-18F254s, Merck) using 0.05 M NH_4HCO_3 :THF (2:3, v/v). Deprotection was accomplished by treatment with 0.2 M anhydrous sodium methoxide in methanol for 30 min at room temperature. The mixture was neutralized with Dowex 50 (H^+) and dried. The final product was stored at -80°C in chloroform:methanol:ammonium hydroxide (2:1:0.003, by vol.). The identity of the product was verified by negative ion MS (electrospray ionization MS; ESI-MS). The mass spectrum was dominated by the M-1 ion of m/z 1060.640, which corresponds to a calculated mass of 1060.641.

Non-microtitre plate enzyme assay. The reaction mixture contained 100 mM TAPS, pH 8.6, 5 mM β -mercaptoethanol, 10 mM MgCl_2 , 30 μM GlcNAc-P-P-DP, 30 μM dTDP-Rha, 1.2 μM dTDP-[^{14}C]Rha (20 000 c.p.m.), 0.1 % *n*-octyl- β -glucopyranoside and 5 μg of membrane proteins in a total volume of 25 μl . GlcNAc-P-P-DP was dried in a test tube and resuspended in 1 % *n*-octyl- β -glucopyranoside by ultrasound (ultrasound bath, 1 min) prior to the addition of other compounds. The reaction was initiated by adding membrane proteins. After incubation at 30°C for 30 min the reaction was quenched by the addition of 1 ml chloroform:methanol (2:1, v/v) and 141 μl water. After separation of the organic layer from the aqueous layer a portion of the organic layer was subjected to scintillation counting. For TLC, the organic layer was dissolved in chloroform:methanol (2:1, v/v) and TLC plates were run in a chloroform:methanol:ammonium hydroxide:water (65:25:0.5:3.5, by vol.) solvent system. Autoradiography was carried using a phosphorimager system.

Liquid chromatography (LC)-MS of Rha-GlcNAc-P-P-DP. LC-MS was performed using an Agilent 6220 TOF spectrometer. A C-18 X-bridge (Waters) HPLC column was used at a flow rate of 0.32 ml min^{-1} . A gradient was run starting with 5 mM ammonium acetate in methanol and progressing linearly to 5 mM ammonium acetate in 20 % hexane in *n*-propanol over 45 min. The column temperature was 45°C . The instrument was operated in an electrospray negative ionization mode (ESI $^-$) and scanned from 180 to 3200 amu. The sample for analysis was prepared as described above with a minor modification. The total volume of the reaction mixture was increased to 100 μl and only 2 μg of membrane protein was used in the reaction. The incubation time was extended to 1 h. The negative control sample was exactly the same as the positive sample, except that GlcNAc-P-P-DP was omitted.

Kinetic studies. For the determination of apparent K_m , substrates were added at various concentrations and protein concentration was reduced to 2 μg . Incubation times were chosen to obtain initial rate data with conversion to product less than 20 %. The apparent K_m values were obtained by a nonlinear regression method using the program Graft 5.0.

To determine whether WbbL follows a ternary complex reaction mechanism or a double-displacement (ping-pong) mechanism, the reaction velocity over a range of dTDP-Rha and GlcNAc-P-P-DP concentrations at several fixed concentrations of both substrates was measured.

Microtitre plate-based assay. The assay for rhamnosyltransferase was performed in 96-well scintillation plates in a 1450 MicroBeta TriLux microplate scintillation and luminescence counter (Perkin-Elmer). The reaction mixture contained 100 mM TAPS, pH 8.6, 5 mM β -mercaptoethanol, 10 mM MgCl_2 , 18 μM GlcNAc-P-P-DP,

35 μM dTDP-Rha, dTDP-[^{14}C]Rha (6000 to 25 000 c.p.m. depending on the experiment), 0.1 % *n*-octyl- β -glucopyranoside and 1 μg of membrane proteins in a total volume of 25 μl . GlcNAc-P-P-DP was dried in a test tube and resuspended in 1 % *n*-octyl- β -glucopyranoside by ultrasound (ultrasound bath, 10 min) prior to the addition of 100 mM TAPS, pH 8.6, 5 mM β -mercaptoethanol, 10 mM MgCl_2 and 1 μg of membrane protein. This mixture (15 μl) was applied to the wells of the microtitre plate. The reaction was initiated by adding the mixture of dTDP-Rha and dTDP-[^{14}C]Rha in buffer (10 μl) and was performed for 15 min at 30°C . The reaction was stopped by adding 100 μl water-saturated butanol and thoroughly mixed 20 times using a multichannel pipette. Then, 75 μl Ready Organic scintillation cocktail (Beckman Coulter) was added.

RESULTS

Cloning and expression of *M. tuberculosis* Rv3265c

The analysis of the sequence of Rv3265c by SOSUI (http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html) indicated that WbbL is a soluble protein, but our data suggested that it is a very tightly associated peripheral membrane protein. After investigation of several systems, expression from pET16b in *E. coli* (ER2566) produced substantial WbbL in the membrane fraction (Fig. 2). The recombinant protein showed a molecular mass consistent with that predicted for Rv3265c-His-tag ($\sim 34\text{ kDa}$). A small amount of enzyme was also expressed in a soluble form (Fig. 2). Soluble and membrane-bound enzyme was assayed using dTDP-[^{14}C]Rha and GlcNAc-P-P-DP in the non-microtitre plate assay, as described above (the synthetic form of GlcNAc-P-P-DP was used in most experiments, because of its greater yield). Both soluble and membrane-bound enzymes were active, but the soluble fraction could not be purified via a nickel-affinity column and was not a practical enzyme source. There was no

Fig. 2. SDS-PAGE analysis of subcellular fractions from *E. coli* expressing Rv3265c (WbbL) visualized by (a) Coomassie brilliant blue and (b) Western blotting. For both (a) and (b), the lanes are: 1, molecular mass markers; 2, membranes; 3, cytosol.

enhancement of soluble enzyme after expression in chaperone-producing strains such as Arctic Express and BL21[DE3]pKJE7. Cloning Rv3265c using the glutathione S-transferase (GST) gene fusion system did not improve solubility in a significant way, nor did solubilization from membranes using detergents and/or salts. Thus, the enzyme was used directly in its membrane-associated form.

The identity of the lipid product produced by the membrane-associated enzyme was checked by TLC. A single band was present that migrated to the same extent as a Rha-GlcNAc-P-P-DP standard (Fig. 3a). Analysis of WbbL lipid product was performed by negative ion electrospray ionization MS (ESI-MS). The standard reaction sample showed the presence of an M-1 ion at m/z 1206.6921, which corresponds to the calculated Rha-GlcNAc-P-P-DP (minus

a proton) mass of 1206.6986 (Fig. 3d, e). This ion was not present in a control sample lacking the acceptor substrate GlcNAc-P-P-DP (Fig. 3b, c).

Determining pH and temperature optima and divalent cation requirements

Preliminary experiments were carried out to determine suitable conditions for studying the kinetics of and developing a microtitre plate-based assay for WbbL. The enzyme showed optimum activity at pH 8.6 and was absolutely dependent on the presence of divalent cations; the addition of 5 mM EDTA abolished enzyme activity almost completely. Enzyme activity was restored by the addition to buffer containing 5 mM EDTA with 20 mM

Fig. 3. TLC and LC-ESI-MS analysis of the WbbL lipid product. (a) TLC analysis of organic soluble material obtained after incubation with WbbL expressed in membranes (lane 1) or cytosol (lane 2) of *E. coli*. A Rha-GlcNAc-P-P-DP standard prepared using *M. smegmatis* membranes (Mikusova *et al.*, 1996) is also shown (lane 3). Organic soluble material obtained after incubation with membranes was analysed by HPLC-MS (electrospray-negative mode). (b, c) The total ion chromatogram (b) and the m/z 1206 ion chromatogram (c) obtained after injection of the control reaction (no GlcNAc-P-P-DP). (d, e) The total ion chromatogram (d) and the m/z 1206 ion chromatogram (e) obtained after injection of the complete reaction mixture. The mass spectral region between m/z 1202 and 1238 is shown as an insert in (d); the ion at m/z 1228 represents the M-2H+Na ion.

Mg^{2+} or 20 mM Mn^{2+} , but not 20 mM Ca^{2+} . The optimum temperature for activity was found to be 30 °C. It was also found that β -mercaptoethanol was required for enzyme activity (data not shown).

Kinetic studies

The kinetic parameters of Rv3265c were investigated using different concentrations of substrates. The apparent K_m for GlcNAc-P-P-DP was found to be 18 μM at 35 μM dTDP-Rha, and the apparent K_m for dTDP-Rha was found to be 35 μM at 18 μM GlcNAc-P-P-DP.

To determine whether WbbL follows a ternary complex reaction mechanism or a double-displacement (ping-pong) mechanism, the reaction velocity over a range of dTDP-Rha and GlcNAc-P-P-DP concentrations at several fixed concentrations of the other substrate was measured (Table 1a, b). For both substrates, the K_m value remained almost the same over a wide range of concentrations of the other substrate, indicating that the enzyme utilizes a ternary complex mechanism in which the two substrates must bind together to the enzyme. Since substrate inhibition was observed above 60 μM for both substrates, kinetic studies were limited to lower concentrations.

Development of a microtitre plate-based assay for rhamnosyltransferase

We have taken advantage of the organic nature of the product to determine enzymic activity by measuring the

incorporation of the radioactive product [^{14}C]Rha-GlcNAc-P-P-DP into a scintillation cocktail (Ready Organic) that is immiscible with aqueous material. We found that by adding butanol to the microtitre plate wells followed by Ready Organic scintillation cocktail, we could get a satisfactory (~67 %) counting efficiency for the Rha-GlcNAc-P-P-DP product, as determined by comparing Ready Organic/butanol microtitre plate counting with counting the product prepared by the standard extraction protocol. The efficiency of counting a known amount of radioactivity in dTDP-[^{14}C]Rha (determined by standard liquid scintillation counting) in the Ready Organic scintillation cocktail mixed with butanol was very low at only ~1.5 %.

The microtitre plate-based assay was run using substrates at their K_m concentrations. To stay in the linear range it was decided to perform a microtitre plate-based assay at 20 % conversion of dTDP-[^{14}C]Rha. We then found that DMSO had no negative effect up to at least 2 % (v/v), and in fact increased the counting efficiency to 80 % (data not shown). We next determined the Z' value (Zhang *et al.*, 1999) for the assay using positive (all components including 1 % DMSO) and background controls (same as positive, except lacking enzyme). These data (Fig. 4a) yielded a Z' value of 0.83, indicating an excellent assay. The assay was further validated by demonstrating the inhibition of WbbL activity by dTDP in a dose-dependent manner (Fig. 4b).

DISCUSSION

WbbL has been classified into family 2 of the GTs with a characteristic fold of superfamily GT-A (<http://www.cazy.org/index.html>). GT-2 enzymes use an inverting mechanism, as does WbbL, which utilizes dTDP- β -Rha as a substrate and generates an α -rhamnosyl product. Given its inverting mechanism it would be expected to catalyse the attachment of the rhamnosyl residue in an SN2-like fashion. Our kinetic evidence is consistent with this mechanism, as it clearly shows that WbbL forms a ternary complex with both substrates being bound. However, detailed kinetic studies were hampered by substrate inhibition. A similar phenomenon was observed by Zhang *et al.* (2006) working with *Bacillus subtilis* TagA, a glycosyltransferase that catalyses a very similar reaction, i.e. the formation of a β -glycosidic bond between *N*-acetylmannosamine and undecaprenyldiphosphoryl-D-*N*-acetylglucosamine (also inversion, in this case an α -sugar nucleotide to a β -linkage).

An earlier assay for enzymes with lipid-linked acceptors uses a solid-liquid bead-based separation system to selectively adsorb the highly hydrophobic products of the reaction (Hyland & Anderson, 2003). In contrast, the microtitre plate-based assay herein is based on the distinction between the organic solubility of the WbbL product, Rha-GlcNAc-P-P-DP, and the aqueous solubility of the dTDP-Rha donor, and importantly no

Table 1. (a) Apparent K_m values for GlcNAc-P-P-DP at the indicated concentrations of dTDP-Rha; (b) apparent K_m values for dTDP-Rha at the indicated concentrations of GlcNAc-P-P-DP

(a) dTDP-Rha concn (μM)*	K_m of GlcNAc-P-P-DP (μM)
20	17 $\pm$ 9
35	18 $\pm$ 4
45	16 $\pm$ 3
50	15 $\pm$ 2
64	15 $\pm$ 4
72	16 $\pm$ 3
(b) GlcNAc-P-P-DP concn (μM)*	K_m of dTDP-Rha (μM)
10	35 $\pm$ 10
18	35 $\pm$ 7
35	36 $\pm$ 7
40	33 $\pm$ 17
50	36 $\pm$ 5
60	34 $\pm$ 3
72	33 $\pm$ 12
80	32 $\pm$ 3
120	36 $\pm$ 12

*Lower and higher concentrations were repeated at least two times.

Fig. 4. Validation of a microtitre plate-based assay for WbbL. (a) The Z' factor determination for the microtitre plate-based assay was done with 20 positive and 20 negative controls (no enzyme). (b) Titration of dTDP. The assay was performed using 18 μ M GlcNAc-P-P-DP, 35 μ M dTDP-Rha, 1.5 μ M dTDP-[14 C]Rha (25 000 c.p.m.) and 1 μ g of membrane proteins.

scintillation beads are required. The key component in transferring from an extraction method to direct and selective counting in an organic scintillation cocktail was the addition of butanol to the reaction mixtures; otherwise, little radioactivity was detected, even when radioactive product was present. The microtitre plate assay shows good reproducibility, and should dramatically aid the detection of WbbL inhibitors in new TB drug discovery efforts. Furthermore, this methodology is readily applicable to other enzymes such as TagA that transfer a glycosyl residue from a sugar nucleotide to a lipid-linked substrate.

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