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In situ activity-based protein profiling of serine hydrolases in *E. coli*

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ABSTRACT

A fluorophosphonate based alkyne activity probe was used for the selective labeling of active serine hydrolases in intact *Escherichia coli* cells. A biotin-azide tag was subsequently attached to the alkyne functionality of the probe with copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. Comparison of proteins from in-cell and lysate labeled preparations suggested qualitatively similar patterns of reactivity in both preparations. Approximately 68%, 30 of the total 44 serine hydrolases detectable in *E. coli* were labeled with the probe indicating significant coverage with a single probe. The methods described here offer a useful tool for profiling and monitoring serine hydrolase activity in situ.

1. Introduction

One of the goals of proteomics is to provide a dynamic picture of protein functionality in the context of the living organism. This requires quantitative and functional assessments of protein status within the cell. Although conventional proteomic analysis provides quantitative or differential expression information relating to compositional changes such approaches do not necessarily report on the activity status of a protein as regulatory mechanisms such as conformational changes and post-translational modifications may not be detected by these methods [1]. Recently activity based protein profiling, ABPP, has been introduced as an approach for simultaneously monitoring the functional states of enzyme families in biological systems [2,3].

Activity profiling is based on the principle that the activity status of an enzyme correlates with the accessibility of

Abbreviations: ABPP, activity based protein profiling; SerHs, serine hydrolase; FP-probe, fluorophosphonate-based probe; THPTA, [tris(3-hydroxy-propyl-triazolyl-methyl)]amine; CuAAC, copper-catalyzed azide-alkyne cycloaddition; TAMRA, carboxytetramethylrhodamine; SDSPAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

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the catalytic site to the substrate [4]. The introduction of substrate incorporating a reactive group targeting the residues of the catalytic site provides a mechanism for covalently linking a probe to those enzymes in an active state. As members of a given enzyme family often employ similar mechanisms of catalysis it is possible to use a single probe to target different members of the family [5]. A single probe can provide the basis for simultaneously monitoring the activities of a many different enzymes in the same family [6].

Members of the serine hydrolase (SerHs) family catalyze a range of reactions including protease, peptidase, esterase, lipase and amidase activities [6]. Thus the monitoring of activities of members of this family with an activity probe can provide information on the status of a diverse set of biochemical processes. Fluorophosphonate-based probes (FP-probes) are highly specific for SerHs. These have been successfully employed for biomarker identification [7,8], analysis of cellular changes in response to viral [9] and fungal infections [10] and for the characterization of serine proteases involved in Drosophila embryogenesis [11].

The use of such probes for in cell labeling of SerHs offers the potential to perform broad scale functional proteomic analysis in the cellular context. Such a development could be a major step in the application of systems based approaches in cell biology. To date most ABPP based applications have employed cell lysates or purified enzymes labeled with SerHs probes. In those few cases where in-cell labeling of serine hydrolase activity was employed differences in reactivity patterns were observed. [12,13]. These differences may arise from reduced probe permeability in living cells, changes in enzyme activity as a result of cell lysis or differences in detection due to inefficiencies of the downstream methods of in cell probe detection. The present studies were undertaken to compare the profiles of serine hydrolase activities labeled in situ or in cell lysates. The rationale being that the disruption of molecular organization associated with cell lysis could alter enzymatic activities which could impact on the subsequent interpretations of the data.

2. Experimental procedures

2.1. Synthesis

Fluorophosphonate alkyne (FP-alkyne) probe ({2-[2-(2-Prop-2-ynyloxy-ethoxy)-ethoxy]-ethyl}-phosphonic acid monoethyl estermonofluoride) and [tris(3-hydroxy-propyl-triazolyl-methyl)]amine (THPTA), the water-soluble ligand for the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction were prepared as previously described [12,14]. Synthetic procedures and a list of reagents and materials are provided in Supplement 1.

2.2. Cell growth and lysis

Escherichia coli K12 Dh5a (Invitrogen) cells transformed with pLKO.1 vector were cultivated in LB media containing Carbenicillin (100 μg/ml). Cells were lysed by sonication in hypo-osmotic Tris-HCl buffer (10 mM, pH 7.4). Cellular debris was removed by centrifugation at 21,000 × *g* for 20 min and the protein concentration in supernatant was determined with a microBCA kit (Pierce).

2.3. SerHs labeling

Biological duplicates were prepared for each labeling condition. For 'in cell' labeling of SerHs, 30 ml cultures of cells in exponential growth (OD600 of 0.7) were incubated for 30 min at 37 °C with 100 μM FP-alkyne probe. The cells were then collected by centrifugation and processed for analysis as described in the following sections. For the "in-lysate" labeling, cell lysates from an equivalent number of cells were incubated with 100 μM FP-alkyne probe for 30 min at 37 °C. "Blank" samples received only equivalent volumes of DMSO, which was the solvent for the probe stock solution. All samples were processed in an identical manner.

2.4. Sample processing

The cell lysates (5 ml each, with 1.3 mg/ml protein) were precleared of endogenous biotin containing materials by incubating with 500 μL of a 50% suspension of streptavidin-agarose beads for 1 h at 4 °C with mixing by rotation. The biotin-azide tag was attached to the alkyne functionality of the probe via copper-catalyzed azide-alkyne cycloaddition for 60 min at room temperature. The cycloaddition conditions were based on the 'optimal' ones described by Finn [14], with the following concentrations of reactants: azide 0.2 mM, Cu(II) 0.41 mM, THPTA-ligand 2.1 mM, aminoguanidine 5 mM and sodium ascorbate 5 mM. Unconjugated probe and biotin tag were removed by 2 buffer exchanges (8 M urea in 100 mM Tris-HCl buffer, pH 8.5) in an Ultracel 10 K cut off unit (Millipore). The samples were then denatured and reduced in 5 ml SDS (0.1%, w/v) and DTT (20 mM) for 30 min at 40 °C. Any protein precipitate which formed after the click-chemistry reaction redissolved during this step. Residual detergent and reducing agent was removed by a single buffer exchange. The samples were then alkylated with IAA (40 mM) for 20 min at room temperature followed by 2 buffer exchanges after which the samples volumes was reduced to 1 ml. Samples were transferred from the filter units to 15 ml vials and diluted with binding buffer (3 M urea, 1% Tween and 250 mM NaCl in Tris-HCl 50 mM pH 8.5.) to a final volume 10 ml. Samples were rotated overnight at 4 °C with 500 μL of a 50% suspension of streptavidin-agarose beads. The beads were washed extensively with urea buffer solution (6 M Urea, Tween 1%, NaCl 250 mM in 50 mM Tris-HCl pH 8.5), then with TFA (0.1%) in water and finally with water. The bound proteins were digested on bead by the addition of 3 μg trypsin in 500 μl of 2 M urea in ammonium bicarbonate buffer (50 mM) with shaking overnight at room temperature.

2.5. Peptides purification

Peptides were lyophilized and resuspended in 1% (v/v) TFA and 2% (v/v) acetonitrile in water and purified by means of RP-HPLC with 200 μL sample injection via a 1 mm × 100 mm analytical column packed with 5 μm Luna C18(2) (Phenomenex, Torrance, CA). Peptides were eluted with a linear 5 min gradient (1–40%) of acetonitrile in water containing 0.1% TFA using

a flow rate of 150 μL/min. Purified peptides were lyophilized and stored at −20 °C. Additional protocols for cell treatment, enzyme labeling and sample preparation are described in supplemental material (Supplement 1).

2.6. Mass spectrometry

Peptides were dissolved in 10 μl of 2% (v/v) acetonitrile, 0.1% (v/v) formic acid (FA) in ultrapure H_2O. Peptides (5 μL sample) were separated on a splitless nanoflow Tempo LC system (Eksigent, Dublin, CA, USA) with injection via a 300 μm × 5 mm PepMap100 precolumn and a 100 μm × 150 mm analytical column packed with 5 μm Luna C18(2) (Phenomenex, Torrance, CA). A 2 h linear gradient 0.33% acetonitrile/min (0–30% B) was used for peptide elution. Both eluents A (2% acetonitrile in water) and B (98% acetonitrile) contained 0.1% formic acid as an ion pairing modifier. Spray voltage was set to 3 kV with a capillary temperature of 150 °C.

Peptides were analyzed using a TripleTOF 5600 quadrupole-time-of-flight hybrid mass spectrometer (AB SCIEX,| Framingham, MA, USA) in standard MS/MS data dependent acquisition mode with a nano-electrospray ionization source. MS survey scans spectra (250 ms) were collected (*m*/*z* 400–1600) followed by 20 MS/MS (100 ms each) on the most intense parent ions (switch criteria: 125 counts/s threshold, +2 to +4 charge state, *m*/*z* 400–1250 mass range), with MS/MS detection range *m*/*z* 100–1600 using the manufacturer's "IDA advanced" settings. Previously targeted parent ions were excluded from repetitive MS/MS acquisition for 12 s (100 ppm mass tolerance).

2.7. Database search and protein identification

Raw spectra WIFF files were processed using standard script (Analyst QS 2.0) to generate text files in Mascot Generic File format (MGF) [15]. MGF files containing the MS/MS spectra information were submitted for protein identification using Global Proteome Machine's X!tandem CYCLONE 2010.12.01.4 search engine (http://50.72.164.137; www.thegpm.org) [16]. Standard Q-TOF settings were used for the search: 100 ppm and 0.4 Da mass tolerance for precursor and fragment ions, respectively; full tryptic specificity with 1 possible missed cleavage were allowed; permitted amino acid modifications included fixed carbamidomethylation of Cys and variable: methionine and tryptophan oxidation/dioxidation, asparagine and glutamine deamidation. A cut-off score of $\log_{10}(e) < -1$ was set for peptides and proteins. The search was restricted to *E. coli* K12 substrain MG 1655. A total of 96,269 *E. coli* K12 sequences were searched using MG1655 uid57779 (NCBI NC_000913.faa 2010.11.22), human (ENSEMBL GRCh37.64) and Common Repository Adventitious Proteins (The GPM 2010.03.24) databases. The search results and spectra can be viewed on the GPM server (http://50.72.164.137; www.thegpm.org). The accession numbers ('lookup model') are GPM77700012901, GPM00300015270 (two replicas for 'in cell' labeled samples), GPM77700012904, GPM77700012905 (two replicas for 'in lysate' labeled samples), GPM77700012906 and GPM77700012909 (two replicas for non-labeled samples) respectively. The false positive rates (FPR) computed by Global Proteome Machine ranged from 0.7 to 0.93% using the programs preset parameters. The data including WIFF, MGF and XML file formats, have been deposited in the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository [17] with the dataset identifier PXD000241. Results for identified peptides and proteins were exported from the Global Proteome Machine (Supplements 2 and 3) with default parameters.

A list of identified serine hydrolase enzymes in the samples was generated by searching the Uniprot and Ecocyc databases for known or predicted serine hydrolases (Supplement 4). This list may not be exhaustive but it includes 49 SerHs that constitute more than 1% of genes in *E. coli* genome.

3. SDS-PAGE separation of the SerHs tagged with TAMRA for fluorescence detection

Aliquots (200 μL/sample) of the FP-alkyne probe labeled 'in cell' or 'in lysate' preparations and DMSO treated control samples, were reacted with TAMRA-azide, 0.2 mM, at room temperature under the same conditions as described above. The labeled samples were separated by 1D SDS PAGE on NuPAGE Bis-Tris precast gels 4–12%, 1.0 mm, 10 well (Invitrogen) according to the manufacturer's protocol.

The TAMRA-tagged proteins were visualized by in-gel fluorescence using a fluorchemQ gel-documentation system (Alpha innotech) using Cy3 channel (λ_{ex} 534 nm, λ_{em} 606) to observe. The gels were subsequently stained with Coomassie blue and scanned to visualize total proteins present in the sample (Fig. 2).

4. 2D LC–MS/MS analysis of proteins in whole cell lysates

E. coli cells were cultured as described above until an OD600 of 1.0 was reached. Cells were harvested by centrifugation, washed twice with cold PBS and lysed with SDS and DTT containing buffer by heating at 95 °C for 5 min. The lysate was sonicated and centrifuged at 16,000 g for 15 min at room temperature. Then lysis buffer was exchanged with 8 M urea on a standard filtration device as published by Winiewski [18] with some method modifications as described in Supplement 1. Proteins were digested with Trypsin (ratio 1:50, Promega) overnight at room temperature and purified with RP-HPLC using a 5 min gradient as described above.

The resulting peptide mixture was adjusted to pH 10 with ammonium formate and separated on a 1 mm × 100 mm XTerra column with a linear water–acetonitrile gradient (20 mM ammonium formate pH 10 in both eluents A and B, 0.66% acetonitrile/min, 150 μL/min flow rate) using an Agilent 1100 Series HPLC system. Forty seven 1-min fractions were collected within the 3–49 min elution window. Samples were concatenated into a total of 22 fractions as described by Dwivedi et al. [19]. Each pool was lyophilized and resuspended in 15 μL of 2% (v/v) acetonitrile, 0.1% (v/v) formic acid (FA) in ultrapure H_2O for the second-dimension separation with LC–MS system. Parameters for LC–MS system were the same as described above with two differences in the settings: gradient slope was set to 0.66% acetonitrile per minute giving 1 h

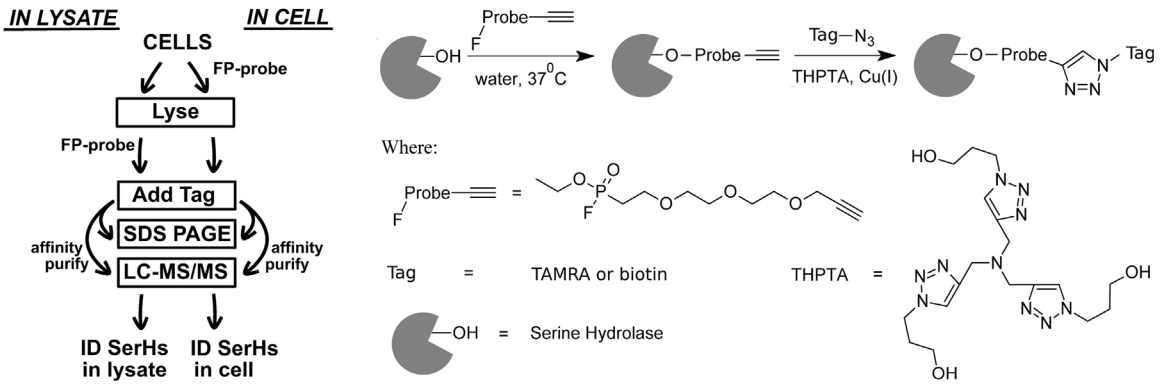

Fig. 1 – Flow chart of experimental design. *E. coli* cells were labeled 'in cell' or lysed and labeled 'in lysate' with a fluorophosphonate-alkyne probe (FP probe) and subsequently reacted with biotin or TAMRA-azide (Tag) via copper-catalyzed azide-alkyne cycloaddition. The TAMRA labeled samples were analyzed for in gel fluorescence following SDS PAGE. The biotin labeled samples purified using a streptavidin affinity column. The serine hydrolases in the bound proteins were identified using LC MS/MS. Details of the copper-catalyzed azide-alkyne cycloaddition are outlined on the left of the figure.

run time and switch criteria threshold was increased to 300 counts per seconds for MS/MS measurements.

Raw spectra WIFF files were treated using standard script (Analyst QS 2.0) to generate text files in Mascot Generic File format (MGF) [15]. MGF files containing the MS/MS spectra information were submitted for protein identification using X!tandem CYCLONE 2013.02.01.2 search engine available in our laboratory as a local version (http://140.193.59.4/tandem/thegpm_tandem.html). The accession number ('lookup model') is GPM22200000195. False positive rate (FPR) computed by Global Proteome Machine was 0.41% with preset parameters. The mass spectrometry proteomics data including WIFF, MGF and XML file formats, have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository [17] with the dataset identifier PXD000241.

5. Results and discussion

This study was designed to compare the serine hydrolase profiles in intact *E. coli* cells with those observed in cell lysates of the same cells using an activity based probe. The intent was to address the question of whether there were quantitative or qualitative differences in the proteins labeled by these two approaches. The rationale being that differences in the labeling patterns or intensities of proteins from the two sources could reflect the need for caution in comparing results derived from these approaches. A flow chart of the experimental design is provided in Fig. 1.

Analysis of the Coomasie blue SDS-PAGE separated proteins derived from samples reacted with the probe 'in cell' or 'in lysate' gave qualitatively and quantitatively identical patterns to control cells treated with DMSO (Fig. 2B). These results indicated that total amounts of protein loaded on the gels were comparable. Furthermore the banding patterns suggested that at the gross level the in cell treatment with the probe had not influenced the protein composition of the cells.

Comparison of the patterns and intensities of 'in cell' or 'in lysate' TAMRA tagged probe reacted proteins indicated some clear differences. The proteins from the 'in cell' labeled samples showed consistently higher levels of fluorescence particularly for some minor bands that were barely detectable in the labeled 'in lysate' samples suggesting that the enzymes were more active in their native intracellular milieu. This was particularly noticeable in the higher molecular weight range proteins despite the fact that Coomasie staining did not indicate any selective protein loss in this region. Given that the relative intensities of bands in the 'in lysate' labeled sample displayed quite variable intensity shifts relative to the 'in cell' sample it appears unlikely that the reduction in was due to lowered labeling efficiency (compare arrowed regions in Fig. 2B). The specificity of the labeling with TAMRA azide tag is noteworthy that in there was virtually no background fluorescence of control cells reacted with the tag (Fig. 2A, lane 1). These results suggested that qualitatively the reactivity patterns of 'in cell' and 'in lysate' labeled proteins were generally comparable.

The above conclusions were based on the premise that the in gel fluorescent bands observed in the same regions of comparator gels (i.e. 'in lysate' vs 'in cell') represent the same protein species. While this may be true in many cases, the resolution of the SDS PAGE is not sufficient to allow such a conclusion. Probe labeled proteins were isolated by affinity chromatography and analyzed by LC MS–MS to directly determine the identities of the labeled proteins in the different samples. The list of identified proteins was then searched against an in house generated list of known or potential *E. coli* serine hydrolase enzymes (see Methods and Materials for details). In total 30 SerHs were identified in one or more of the analyses (i.e. 'in cell', 'in lysate' or DMSO treated control) of these 28 were detected in both 'in cell' and 'in lysate' preparations. The D-alanyl-D-alanine carboxypeptidase, dacB, was only confidently detected in the 'in cell' labeled sample while the periplasmic serine endoprotease, degS, was observed exclusively in the 'in lysate' preparations (Table 1). Based on these results it appeared that the same enzymes were labeled

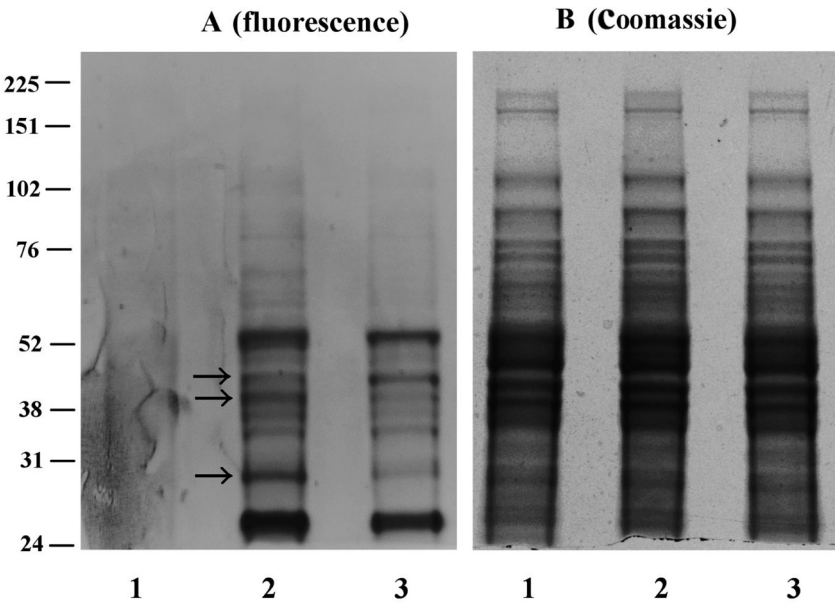

Fig. 2 – Gel views of *E. coli* proteins and FP-TAMRA labeled Proteins. (A) Proteins from (1) a non-labeled control blank cell preparation or from fluorophosphonate-alkyne probe (2) 'in cell' or (3) 'in lysate' labeled preparations were separated by SDS PAGE and the fluorescent proteins were visualized. Arrows indicate examples of qualitative and quantitative differences in labeling. (B) The same gels were subsequently stained with Coomassie blue. The gels display similar patterns and of protein expression and indicate similar quantities of protein loading on the gels.

and isolated using the different labeling conditions. In contrast 10 SerHs were detected in biotin azide labeled control preparations. The MS/MS signal intensity, log(*I*), of those peptides from the SerHs isolated in the controls were at least 2 logs lower than those for the same proteins identified in the labeled samples. Similarly the numbers of unique peptides per SerHs in the control were less than half of those observed in the 'in cell' and 'in lysate' preparations raising the possibility that suggested that the SerHs identified in the control isolates were trace contaminants of high abundance SerHs.

The repertoire of SerHs actually expressed in *E. coli* A in a whole cell lysate of *E. coli* was examined by 2D-LC–MS. In contrast to the above probe labeled studies these analyses provided information on the presence of proteins but not on the activity of these enzymes. Quite extensive coverage of the *E. coli* proteome was obtained with identification of 2496 proteins with two or more unique peptides, representing 60% from 4141 proteins coded in genome. A total of 44 possible serine hydrolase enzymes were detected (Supplement 6) suggesting that approximately 32% (14 of 44) of these enzyme species had not been detected using the probe ABPP. There are several possible explanations for these results. The most obvious is that enzymes were present but not active at the time of analysis. Alternatively some of the SerHs may not react with this probe. Structural features of the probe, particularly the linker, can markedly influence the reactivity profile. Thus a single probe is not expected to detect all members of the serine hydrolase family because of the structural diversity of the substrates employed by these enzymes. This last possibility could potentially be dealt with by using a mixture of SerHs probes with different linkers to increase the potential coverage of the labeled.

It was somewhat unexpected to observe that that the SerHs identified in the two samples by MS/MS were quantitatively and qualitatively so similar. Although it might be argued that based on peptide signal intensity there was a trend for higher SerHs abundances in the 'in cell' labeled preparation, in our opinion it was not very compelling. This raises a question of the relationship between the data derived from the in gel and MS/MS comparisons of the samples. Samples for both of these applications were labeled with probe under detergent free conditions in order to maintain enzyme activity. However they were subsequently processed differently. The samples for SDS PAGE were dissolved in sample buffer containing detergent. While the samples for MS/MS were prepared in detergent free buffer. Thus it may be that the SerHs for the latter were not fully extracted.

The fluorophosphonate probe used in these studies covalently modifies the active site SerHs and irreversibly inactivates them. This raised concerns regarding the physiological effects of the 'in cell' labeling procedure. However, there was no apparent effect on the exponential growth rate of the treated cells under the 30 min labeling conditions employed. Furthermore there was no impact on the final cell densities that were achieved. These results suggested either that the probe concentration was not saturating for target enzymes in culture or that the SerHs activities were not essential for cell survival. It was also conceivable that the rate of de novo enzyme replacement may be sufficient to sustain cellular activity.

The results of these studies suggest that the probe and labeling conditions described here provide an effective means of in situ labeling active serine hydrolases. Our modified procedure increased the efficiency of probe detection relative

Table 1 – Serine hydrolases identified in *E. coli*.

N	Protein name	Gene name	Accession	EC number	log *I*	Peptides
1	Lon protease	lon	P0A9M0	EC 3.4.21.53	6.59/5.03	62/15
2	ATP-dependent Clp protease proteolytic subunit	clpP	P0A6G7	EC 3.4.21.92	7.17/5.62	35/5
3	Probable protease sohB	sohB	P0AG14	EC 3.4.21.-	4.92/3.80	8/2
4	Uncharacterized protein yjjU	YjjU	P39407	EC 3.1.1.-	6.25/5.52	20/12
5	Putative esterase YheT	yheT	P45524	EC 3.1.1.-	5.63/5.06	16/8
6	Murein tetrapeptide carboxypeptidase	ldcA	P76008	EC 3.4.17.13	6.44/6.01	24/16
7	Tail-specific protease	prc	P23865	EC 3.4.21.102	4.23/3.84	5/2
8	D-alanyl-D-alanine carboxypeptidase dacB	dacB	P24228	EC 3.4.16.4	3.41/0	2/0
9	Protease 4	sppA	P08395	EC 3.4.21.-	5.72/5.47	17/15
10	Esterase yqiA	yqiA	P0A8Z7	EC 3.1.-	5.95/5.75	7/5
11	Lysophospholipase L2	pldB	P07000	EC 3.1.-	5.94/5.79	23/19
12	NTE family protein rssA	rssA	P0AFR0		5.66/5.52	12/13
13	Beta-lactamase	ampC	P00811	EC 3.5.2.6	5.41/5.28	14/11
14	Esterase YpfH	ypfH	P76561	EC 3.1.-	6.25/6.14	5/6
15	Acyl-CoA thioesterase I	tesA	P0ADA1	EC 3.1.2.-	6.52/6.41	10/10
16	Esterase yjfP	yjfP	P39298	EC 3.1.-.-	6.34/6.25	18/15
17	Esterase ybfF	ybfF	P75736	EC 3.1.-	7.00/6.96	34/34
18	Protease 2	ptrB	P24555	EC 3.4.21.83	5.93/5.90	26/25
19	Acetyl esterase	aes	P23872	EC 3.1.1.-	5.49/5.54	13/13
20	Probable KDGal aldolase YagE	yagE	P75682	EC 4.1.2.-	5.86/6.02	14/14
21	D-alanyl-D-alanine carboxypeptidase dacA	dacA	P0AEB2	EC 3.4.16.4	6.20/6.50	22/26
22	Periplasmic pH-dependent serine endoprotease DegQ	degQ	P39099	EC 3.4.21.107	6.46/6.82	30/35
23	Pimelyl-[acyl-carrier protein] methyl ester esterase	bioH	P13001	EC 3.1.1.85	5.28/5.67	7/10
24	UPF0214 protein yfeW	yfeW	P77619		4.15/4.63	3/4
25	D-Alanyl-D-alanine carboxypeptidase dacC	dacC	P08506	EC 3.4.16.4	5.69/6.21	18/22
26	ATP-dependent Clp protease ATP-binding subunit ClpA	clpA	P0ABH9		3.50/4.06	1/3
27	Periplasmic serine endoprotease DegP	degP	P0C0V0	EC 3.4.21.107	6.83/7.59	35/56
28	S-formylglutathione hydrolase frmB	frmB	P51025	EC 3.1.2.12	4.14/5.47	2/10
29	S-formylglutathione hydrolase yeiG	yeiG	P33018	EC 3.1.2.12	3.64/6.06	2/16
30	Serine endoprotease DegS	degS	P0AEE3	EC 3.4.21.107	0/5.36	0/11

Proteins were labeled with a fluorophosphonate-alkyne probe either 'in cell' or 'in lysate'. Samples pre cleared of endogenous biotin with streptavidin-agarose and then reacted with a biotin-azide tag via copper-catalyzed azide-alkyne cycloaddition. The boitinylated proteins were concentrated with streptavidin-agarose beads, washed and digested on the bead with trypsin. Samples were analyzed by LC-MS/MS and the proteins were searched against a list of known or predicted *E. coli* serine hydrolase enzymes. The column labeled "log *I*" gives the ratios of protein intensities calculated using the sums of the intensities of the fragment ion spectra for the corresponding proteins in both samples (i.e. 'in lysate' and 'in cell'). The column labeled "Peptides" gives the ratios of the numbers of peptides identified for the corresponding proteins in both samples. In both cases the values represent in cell/in lysate ratios of duplicate samples. Values for protein intensities and unique peptide numbers were obtained with X!tandem search engine.

to previously described methods [12]. The 'in cell' labeling provided similar SerHs coverage to that obtained by 'in cell' labeling with the probe. This suggested that the probe readily enters the cell and reacts with the serine hydrolases in situ. This study provides one of the first cases in which such high levels of in cell labeling have been achieved for SerHs. The availability of such capabilities offers many exciting possibilities for the analysis of activity changes relating to physiological responses. The approach may be of relevance to the characterization of organisms, particularly those requiring thermophilic or anaerobic growth conditions where disruption of the cellular environment may lead to marked alterations in enzymatic activity. Equally relevant is the capacity to use the probes to monitor novel uncharacterized organisms as a tool for functionally annotating their protein functions. The serine hydrolases offer a particularly relevant area of analysis because of the diverse types of reactions that members of this family catalyze. The fact that the members of this family account for more than 1% of *E. coli* genes suggests a central role for their activities. The capacity to perform live cell monitoring offers many new opportunities to dynamically monitor the roles of serine hydrolase activities in cellular metabolism under a variety of physiological conditions.

Conflict of interest

The authors have no conflicts of interest to declare.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.euprot.2014.04.007.

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