Analysis of Steady-state Protein Phosphorylation in Mitochondria Using a Novel Fluorescent Phosphosensor Dye*

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The phosphorylation of mitochondrial proteins is pivotal to the regulation of respiratory activity in the cell and to signaling pathways leading to apoptosis, as well as for other vital mitochondrial processes. A number of protein kinases have been identified in mitochondria but the physiological substrates for many of these remain unknown or poorly understood. By necessity, most studies of mitochondrial phosphoproteins to date have been conducted using in vitro incorporation of 32P. However, proteins that are highly phosphorylated from in situ reactions are not necessarily detected by this approach. In this study, a new small molecule fluorophore has been employed to characterize steady-state levels of mitochondrial phosphoproteins. The dye is capable of sensitive detection of phosphorylated amino acid residues in proteins separated by gel electrophoresis. When the fluorescent dye is combined with a total protein stain in a sequential gel staining procedure, the phosphorylated proteins can be visualized in the same gel as the total proteins. To optimize resolution of the proteins in mitochondria, a previously described sucrose gradient fractionation method was employed prior to gel electrophoresis. Phosphorylated proteins, as defined by the fluorescence of the phosphosensor, were excised from the gels and identified by peptide mass fingerprinting. One novel and prominent phosphoprotein identified in this manner was determined to be the 42-kDa subunit of mitochondrial complex I.

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A number of protein kinases are known to be localized within mitochondria, including pyruvate dehydrogenase kinase, branched-chain α-ketoacid dehydrogenase kinase, cAMP-dependent protein kinase, protein kinase Cβ, stress-activated protein kinase, and A-Raf, as well as an unidentified tyrosine kinase (1, 2). Determination of the physiological substrates of many of these kinases has proved to be challenging. Global analysis of mitochondrial phosphoproteins has to date been performed by incubating isolated mitochondria with [γ-32P]ATP (2–9) or by labeling cells cultured in phosphate-free medium by guest on July 23, 2018http://www.jbc.org/Downloaded from

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¶ The abbreviations used are: MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; ANT, adenine nucleotide translocase.

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g for 20 min at 4 °C (TLA100.2 rotor, Beckman-Coulter, Fullerton, CA) and resuspended at a protein concentration of 5 mg/ml with 100 mM Tris/HCl, 1 mM EDTA, pH 7.5, 1 μg/ml pepstatin, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 1% lauryl maltoside, as well as 1/100 volume phosphatase inhibitor mixtures 1 and 2 (Sigma). Mitochondria were incubated in the solution for 20 min on ice with stirring, before the extracted membranes were pelleted at 174,000 × g for 20 min at 4 °C. The resulting supernatant was then layered on top of 4.5 ml of a 15–35% sucrose step gradient. The gradient was centrifuged for 16.5 h at 128,000 × g at 4 °C using an SW 50.1 rotor. The sucrose gradient was fractionated into 500-μl aliquots, which were frozen at −80 °C. Proteins were quantified by the bicinchoninic acid (BCA) solution assay using bovine serum albumin as the protein standard (22).

**Phosphoprotein Separation and Detection Procedures—**SDS-polyacrylamide gel electrophoresis was performed by standard methods (23). Proteins were concentrated using a chloroform/methanol precipitation procedure (24) before resuspension in sample buffer and heating for 5 min at 95 °C. Samples were cooled to room temperature before gel loading and electrophoresis. For two-dimensional gel electrophoresis, mitochondrial proteins were prepared as described previously (18). All samples were precipitated before two-dimensional gel electrophoresis to minimize unspecific staining due to phospholipids and other cell constituents. Approximately 100–150 μg of protein was separated for 80,000 V-h on pH 3–10 Immobiline Drystrip-immobilized pH gradient gels (Amersham Biosciences). After isoelectric focusing, SDS-polyacrylamide gel electrophoresis was performed using an Investigator two-dimensional system (Genomic Solutions, Ann Arbor, MI). Fluorescent staining of SDS-polyacrylamide gels using Pro-Q Diamond phosphoprotein gel stain (Molecular Probes, Eugene, OR) was performed by fixing the gels in 45% methanol, 5% acetic acid overnight, washing with three changes of deionized water for 10 to 20 min per wash, followed by incubation in Pro-Q Diamond phosphoprotein gel stain for 180 min, and destaining with successive washes of 15% 1,2-propanediol or 4% acetonitrile in 50 mM sodium acetate, pH 4.0. Useful images could be obtained 3 h after staining, employing three successive destaining washes. Following image acquisition, gels were stained for total protein with SYPRO Ruby protein gel stain (Molecular Probes) for serial dichromatic detection, permitting comparison of phosphoprotein and total protein profiles (20, 21).

Images were acquired on a Fuji FLA 3000 laser scanner (Fuji Photo Film Co., Ltd., Tokyo, Japan) with 532 nm excitation and 580 nm band pass emission filter for Pro-Q Diamond dye detection and with 473 nm excitation and 580 nm band pass emission filter for SYPRO Ruby dye detection. For two-dimensional gels, computer-generated differential display maps of protein phosphorylation and protein expression patterns were generated using Z3 software (Compugen, Tel-Aviv, Israel) (25). With this system, spots from the reference gel appear green and those that differ in intensity levels appear magenta. This facilitates identification of differentially expressed protein spots by simple visual inspection.

**Matrix-assisted Laser Desorption Time-of-Flight Mass Spectrometry—**After detecting proteins in polyacrylamide gels with Pro-Q Diamond phosphoprotein gel stain and staining with SYPRO Ruby protein gel stain, protein bands were subjected to trypsin digestion and mass spectrometry, as described previously (19, 26). Mass spectrometry was performed using an Axima CFR MALDI-TOF mass spectrometer (Kratos Analytical, Chestnut Ridge, NY) with an accelerating voltage of 20 kV, and profiles were internally calibrated using trypsin auto-proteolytic fragments. Peptide-mass fingerprinting data were evaluated using the Kratos Launchpad analysis package and the Protein Prospector database.

**Western Blotting—**Large format one-dimensional gels were electroblotted according to published protocols (27). Phosphoamino acid-specific antibodies were visualized by chemiluminescence using an ECL kit (Amersham Biosciences). The anti-phosphoserine antibody was from Zymed Laboratories Inc. (South San Francisco, CA), whereas the anti-phosphothreonine and anti-phosphotyrosine antibodies were from Cell Signaling Technology (Beverly, MA).

**RESULTS AND DISCUSSION**

**Multiplexed Proteomics Analysis of Steady-state Protein Phosphorylation—**A novel fluorescent phosphorylation sensor, referred to as Pro-Q Diamond dye, was recently described that is capable of detecting phosphorylated proteins in polyacrylamide gels (19). This study utilizes the technology to analyze the steady-state levels of phosphoproteins in mitochondria. Pro-Q Diamond dye has been shown to discriminate between phosphorylated and unphosphorylated proteins with a high specific-to-nonspecific staining ratio using several model proteins (19). However, in cells or organelles, the dynamic range of protein abundance can span six orders of magnitude. For this reason it is important to define the total protein profile, as was done in this case using SYPRO Ruby dye. The ratio of the Pro-Q Diamond dye signal to the SYPRO Ruby dye signal provides a measure of the phosphorylation levels with respect to amounts of each protein in the sample. Since both dyes bind to proteins noncovalently, phosphoproteins may subsequently be identified by standard peptide mass profiling procedures.

Fig. 1 shows a representative SDS-polyacrylamide gel of the sucrose gradient fractions 1–9, as well as unfractionated bovine heart mitochondria, stained with Pro-Q Diamond dye (Fig. 1A) and then subsequently with SYPRO Ruby dye (Fig. 1B). Several proteins are obviously stained with the phosphoprotein-selective dye, and these proteins do not in general correspond to the most abundant proteins in the gradient fractions. As an
example of the differential staining, the gel lane containing fraction 2 of the sucrose gradient was investigated further using image analysis software. Electrophoretic profiles were obtained for the lane after Pro-Q Diamond dye staining and then after SYPRO Ruby dye staining. The overlay of the two profiles (Fig. 2) demonstrates that a 40-kDa protein is stained significantly by the phosphate-selective dye. There was little or no staining of the other proteins present in this lane, confirming the earlier studies showing that the background of nonspecific labeling of unphosphorylated proteins is low (19). The treatment of phosphoproteins or phosphopeptides with a strong base (0.1 M Ba(OH)$_2$) eliminates phosphoric acid from phosphoserine and phosphothreonine residues through a β-elimination reaction (28).

Elimination experiments confirmed phosphorylation of the protein from gradient fraction 2. Using in-gel barium hydroxide treatment at 37°C for 1 h, led to loss of up to 50% of the Pro-Q Diamond dye binding to the 40-kDa protein compared with an untreated control gel, whereas no Pro-Q Diamond signal was obtained from ovalbumin after β-elimination of the serine phosphate residues (data not shown).

As a first operational screen, a Pro-Q Diamond dye-to-SYPRO Ruby dye fluorescence ratio (D/S) that was 1.5 times the averaged ratio obtained with the nonphosphorylated molecular mass markers bovine serum albumin, phosphorylase b, carbonic anhydrase, and β-galactosidase (in this case 0.36) was used to define phosphoproteins in gels of sucrose gradient fractionated mitochondrial extracts. All ratios were corrected for molecular mass, since a protein with 100 kDa mass will bind more SYPRO Ruby dye molecules than a 50-kDa protein and result in a 50% lower D/S ratio for the larger protein. By the cited criterion, there were five prominent phosphoproteins revealed in the SDS-polyacrylamide gel analysis indicated in Fig. 1.

Identification of the 42-kDa Protein as a Novel Phosphoprotein in Complex I—To further investigate the phosphorylation of the ~40-kDa protein, sucrose gradient fraction 2 was separated by two-dimensional gel electrophoresis, the spots excised, digested with trypsin, and subjected to mass spectrometry analysis. The peptide mass profile identified the protein unequivocally as the bovine homologue of human NDUFA10 of Complex I, the NADH:ubiquinone oxidoreductase. Complex I catalyzes the first step of the electron transport chain, the transfer of two electrons from NADH to ubiquinone, coupled to the translocation of four electrons across the membrane. It is found in the inner mitochondrial membrane as an assembly with molecular mass of over 900 kDa and consists of ~46

**FIG. 2.** Fluorescence intensity profile of the gel lane corresponding to sucrose gradient fraction 2 obtained after staining with Pro-Q Diamond dye (black trace) or SYPRO Ruby dye (gray trace). The respective lanes from the gel are shown to the right of the line traces (PD = Pro-Q Diamond dye, SR = SYPRO Ruby dye). One prominent phosphoprotein was observed.

**FIG. 3.** Characterization of NDUFA10 isoforms by two-dimensional gel electrophoresis of mitochondrial sucrose gradient fraction 2. A, computer-generated overlay of the gels after Pro-Q Diamond dye staining and SYPRO Ruby dye staining. Phosphoproteins appear magenta in the image, while nonphosphorylated proteins appear green. Where the signals overlap, the spots appear gray. B, enlarged area showing the NDUFA10 protein only. C, Pro-Q Diamond dye signal/SYPRO Ruby dye signal ratios (D/S) for the five spots shown in B.

**FIG. 4.** Western blot analysis of NDUFA10 using three different phosphoamino acid-specific antibodies, as indicated to the left of each image. Detection was performed by chemiluminescence using a horseradish peroxidase-conjugated secondary antibody. The analysis indicates that NDUFA10 contains phosphothreonine residues, but not phosphoserine or phosphotyrosine residues.
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| Protein                | Ratio (D/S) | Molecular mass<sup>a</sup> | Ratio (D/S) molecular mass corr.<sup>b</sup> | p<sup>c</sup> | MOWSE          | Accession numbers<sup>d</sup> |
|-----------------------|------------|----------------------------|---------------------------------------------|----------|----------------|-----------------------------|
| ANI + 3               | 8.81       | 32.83                      | 7.88                                        | 9.84     | 9.4 × 10<sup>6</sup> | P02722(399011)               |
| CI-NDUFA10            | 2.44       | 36.69                      | 2.44                                        | 5.83     | 3.2 × 10<sup>6</sup> | P34942(464254)               |
| NAD(P) transhydrogenase | 0.2        | 109.2                      | 0.59                                        | 7.29     | 2.55 × 10<sup>6</sup> | P11024(128400)               |
| Aconitase             | 0.23       | 82.4                       | 0.52                                        | 7.41     | 1.7 × 10<sup>2</sup> | P20004(27808769)            |
| Phosphate-carrier protein | 0.25   | 35.02                      | 0.51                                        | 9.23     | 1.30 × 10<sup>6</sup> | P12334(627754)               |
| SDH-Fp                | 0.25       | 68.32                      | 0.46                                        | 6.39     | 2.0 × 10<sup>6</sup> | P10393(264685)               |
| Isocitrate dehydrogenase | 0.32     | 46.51                      | 0.41                                        | 8.5      | 1.6 × 10<sup>6</sup> | Q04467(417178)               |
| CIII-Core I           | 0.22       | 49.21                      | 0.29                                        | 5.46     | 5.6 × 10<sup>2</sup> | P31800(27807137)            |
| Creatine kinase (basic) human | 0.22 | 43.35                      | 0.26                                        | 7.28     | 1.9 × 10<sup>-1</sup> | P17540(26010521)            |
| CIII-core II          | 0.19       | 46.52                      | 0.24                                        | 7.79     | 2.4 × 10<sup>-3</sup> | P35004(27871433)            |
| CV-β                  | 0.16       | 51.56                      | 0.22                                        | 5.0      | 7.7 × 10<sup>-4</sup> | P90629(114545)               |
| CV-α                  | 0.1        | 55.26                      | 0.15                                        | 8.27     | 1.4 × 10<sup>-2</sup> | P19483(27807237)            |
| PDH E1-α              | 0.31       | NA                         | NA                                          | NA       | NA             | NA                          |

<sup>a</sup> Molecular mass in kilodalton of the mature protein.
<sup>b</sup> The Pro-Q Diamond dye signals/SYPRO Ruby dye signals were normalized to the NDUFA10 molecular mass.
<sup>c</sup> Isoelectric point of the mature protein.
<sup>d</sup> Accession numbers are according to Swiss-Prot and NCBI protein databases.

Subunits (29, 30), seven being encoded by mitochondrial DNA, while the remaining are encoded by nuclear DNA.

The identification of NDUFA10 was further confirmed and extended in two-dimensional gel electrophoresis experiments. The phosphorylated protein migrated to a similar position as the 42-kDa protein (NDUFA10) annotated on a previously published map of bovine complex I (29). As shown in Fig. 3, SYPRO Ruby dye staining revealed five isoforms for the protein (spots 1–5), three of which were phosphorylated, based upon Pro-Q Diamond dye staining. The most straightforward interpretation of the staining patterns of the NDUFA10 isoforms is as follows: spots 1 and 2 are two unphosphorylated forms, different in charge through amino acid differences or post-translational modifications other than phosphorylation. Spots 3 and 4 are the phosphorylated forms of each or are the mono- and diphosphorylated forms of one of them. Spot 5 is a phosphorylated form of a third variant of the polypeptide, whose nonphosphorylated form is buried in spot 4. Notably, the intensity ratio of phosphate-to-protein in spot 4 is close to twice that in spot 3. As the Pro-Q Diamond dye signal intensity correlates with the number of phosphate residues on a protein (19), it appears that the isoform in spot 4 is doubly phosphorylated and in spot 3 singly phosphorylated. Based upon Western blot analysis (Fig. 4) NDUFA10 contains only phosphothreonine residues, while a cAMP phosphorylation motif scan of the polypeptide sequence using the ProSite data base program (31) identified two threonine phosphorylation motifs, KKM and KKVT. No potential sites of protein kinase A-mediated serine phosphorylation were uncovered by this analysis.

The 18-kDa subunit of complex I (human NDUFS 4) has previously been reported to be phosphorylated by a cAMP-dependent protein kinase using exogenously added kinase and [γ-<sup>32</sup>P]ATP or by anti-phosphoserine immunoblottedting of proteins from mitoplasts of activated cells (5–7, 32). Phosphorylation of this subunit was not observed here using the described Multiplexed Proteomics technology (see Fig. 2), suggesting that the protein is not typically phosphorylated to a significant extent in resting mitochondria.

Other proteins besides NDUFA10 for which the ratio of Pro-Q Diamond dye staining to SYPRO Ruby dye staining was more than 1.5 times the average background are listed in Table I. These are adenine nucleotide translocase (ANT), isocitrate dehydrogenase, flavoprotein of succinate dehydrogenase, NAD(P) transhydrogenase, aconitase, and the phosphatase carrier protein. The other proteins that reacted significantly with the Pro-Q Diamond stain, but did not produce the 1.5 ratio of phosphate to protein stain used in the first cut off, are listed in Table I. These include α and β subunits of complex V, core proteins I and II of complex III, and creatine kinase. A phosphoprotein of ~40 kDa in sucrose gradient fractions 7 and 8 was identified by monoclonal antibody reaction as the α subunit of pyruvate dehydrogenase, but the identity of this protein could not be confirmed by mass spectrometry, probably because the bovine sequence is significantly different from the human sequence used in the analysis. This latter group of proteins probably represents those for which only a fraction of the total are phosphorylated in the steady state under conditions present in heart tissue. It is important to note that some of the proteins listed in Table I have previously been identified as phosphoproteins (9, 10). However, the major phosphoproteins described here, NDUFA10 and ANT, have not been reported previously.

In summary, we describe the first use of multiplexed proteomics technology on a complex mixture of proteins, bovine heart mitochondria, and establish the utility of the protocols for identifying phosphoproteins in one-dimensional and two-dimensional gels. Importantly it is clearly possible to distinguish between phosphorylation of the different isoforms of a polypeptide with this technology. Here we focus on NDUFA10 of Complex I, whose identification as a phosphoprotein is novel and intriguing. The phosphorylation of this subunit could serve two distinct functions. One might be to control the activity of Complex I through binding of NADH (33). Additionally, since it has been noted that the NDUFA10 subunit of Complex I is easily lost during purification of complex I, the phosphorylation might influence the binding affinity of NDUFA10 and in turn regulate the amount of fully active Complex I in the inner-membrane of mitochondria.

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REFERENCES
1. Thomson, M. (2002) Cell. Mol. Life Sci. 59, 213–219
2. Salvi, M., Bruniati, A., Bordin, L., La Rocca, N., Clari, G., and Toninello, A. (2002) Biochim. Biophys. Acta 1589, 181–185
3. Piedimonte, G., Silvotti, L., Chamaret, S., Borghetti, A., and Montagnier, L. (1986) J. Cell. Biochem. 32, 113–123
4. Technikova-Dobrova, Z., Sardanelli, A. M., Stanca, M. R., and Papa, S. (1994) FEMS Lett. 350, 187–191
5. Sardanelli, A. M., Technikova-Dobrova, Z., Scacco, S. C., Speranza, F., and Papa, S. (1995) FEMS Lett. 377, 470–474
6. Sardanelli, A. M., Technikova-Dobrova, Z., Speranza, F., Mazzocca, A., Scacco, S., and Papa, S. (1996) FEMS Lett. 396, 276–278
7. Technikova-Dobrova, Z., Sardanelli, A. M., Scacco, S., Signorile, A., Lorusso, V., and Papa, S. (2001) Biochemistry 40, 13941–13947
8. Corso, M., and Thomson, M. (2001) Placenta 22, 432–439
9. Bykova, N., Egsgaard, H., and Møller, I. (2003) FEBS Lett. 540, 141–146
10. Hojlund, K., Wrzesinski, K., Mose Larsen, P., Fey, S. J., Roepstorff, P., Handberg, A., Dela, F., Vinten, J., McCormack, J. G., Reynet, C., Beck-Nielsen, H. (2003) J. Biol. Chem. 278, 10436–10442
11. Yeargin, J., and Hass, M. (1995) Curr. Biol. 5, 423–431
12. Hu, V., and Heikka, D. (2000) FASEB J. 14, 423–431
13. Hu, V., Heikka, D., Dieffenbach, P., and Ha, L. (2000) FASEB J. 14, 1562–1568
14. Hansson, B. J., Schulenberg, B., Patton, W. F., and Capaldi, R. A. (2001) Electrophoresis 22, 950–959
15. Taylor, S. W., Fahy, E., Zhang, B., Glenn, G. M., Warnock, D. E., Wiley, S., Murphy, A. N., Gaucher, S. P., Capaldi, R. A., Gibson, B. W., Ghosh, S. S. (2003) Nat. Biotechnol. 21, 281–286
16. Patton, W., and Beechem, J. (2002) Curr. Opin. Chem. Biol. 6, 63–69
17. Steinberg, T., Agnew, B. G., Leung, W., Goodman, T., Schulenberg, B., Hendrickson, J., Beechem, J., Haugland, R., and Patton, W. (2003) Proteomics, in press
18. Hart, C., Schulenberg, B., Dwez, Z., Leung, W. Y., and Patton, W. F. (2003) Electrophoresis 24, 599–610
19. Wessel, D., and Flugge, U. I. (1984) Anal. Biochem. 138, 141–143
20. Wessel, D., and Flugge, U. I. (1984) Anal. Biochem. 138, 141–143
21. Wessel, D., and Flugge, U. I. (1984) Anal. Biochem. 138, 141–143
22. Wessel, D., and Flugge, U. I. (1984) Anal. Biochem. 138, 141–143
23. Wessel, D., and Flugge, U. I. (1984) Anal. Biochem. 138, 141–143
24. Wessel, D., and Flugge, U. I. (1984) Anal. Biochem. 138, 141–143
25. Smilansky, Z. (2001) Electrophoresis 22, 1616–1626
26. Aggeler, R., Coons, J., Taylor, S. W., Ghosh, S. S., Garcia, J. J., Capaldi, R. A., and Matusch, M. F. (2001) J. Biol. Chem. 277, 33906–33912
27. Steinberg, T., Agnew, B. G., Leung, W., Goodman, T., Schulenberg, B., Dwez, Z., Leung, W. Y., and Patton, W. F. (2003) Electrophoresis 24, 599–610
28. Byford, M. (1991) Biochem. J. 260, 261–265
29. Carroll, J., Shannon, R. J., Fearnley, I. M., Walker, J. E., and Hirst, J. (2002) J. Biol. Chem. 277, 50311–50317
30. Byford, M. (1991) Biochem. J. 260, 261–265
31. Byford, M. (1991) Biochem. J. 260, 261–265
32. Byford, M. (1991) Biochem. J. 260, 261–265
33. Yamaguchi, M., Belogrudov, G. I., Matsuno-Yagi, A., and Hatefi, Y. (2000) Eur. J. Biochem. (Tokyo) 267, 329–336
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