

Detection of redox modified proteins by gel-free proteomics

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Introduction

Although cysteines are one of the most rarely used amino acids, they are usually highly conserved and can play crucial roles in the structure and function of proteins (Pe'er et al., 2004). The reactive thiol group may undergo a number of modifications such as nitrosylation, oxidation to sulfenic (-SOH), sulfinic (-SO₂H) or sulfonic (-SO₃H) and involvement in disulfide bonds. The redox state of a protein's thiol groups can influence the protein's structure and function and form the molecular basis of redox control of many proteins (Leichert and Jakob, 2006). It is widely regarded that the formation of disulfide bonds including glutathiolation, is a form of protection in the presence of oxidants and conditions of oxidative stress (OS). Reversible changes may be a means of protection from further irreversible oxidation which would result in protein degradation (Ying et al., 2007).

Material and methods

Protein extracts from exponentially growing *Saccharomyces cerevisiae* ($A_{600} = 2-3$) exposed to 100 μM H₂O₂ for one hour were used for initial experiments. Proteins involved in disulfides were detected by a "Biotin Switch" method adapted from (Poole et al., 2004), except 100mM NEM was used instead of 50mM IAM in the lysis buffer. Proteins were separated by 1D electrophoresis, numbered bands were excised and tryptic digested for analysis by MALDI-TOF. For gel-free identification of proteins, biotinylated peptides were tryptic digested before avidin capture. Peptides were released by mercaptoethanol and subjected to LC-MS/MS.

Results

Proteins identified by MALDI-TOF in Figure 1 are known to contain cysteines and have been

reported as been involved in disulfide bonds. Lanes 3 and 4, show precipitated proteins after first wash of avidin beads i.e. proteins not bound to avidin. Lanes 5 and 6 indicate there are no unbound proteins after final wash and the method is selective for proteins involved in disulfide bonds. Proteins consistently identified by the gel-free method include those identified by MALDI-TOF. In general a larger population of proteins were identified as containing disulfides in yeast treated with H₂O₂.

The major goal in identifying redox regulated proteins involves determining which proteins are involved, which cysteines within those proteins are most reactive and identifying the thiol modification itself (Ying et al., 2007). In the gel-free identification, release of biotinylated peptides by mercaptoethanol allows the identification of the Cys involved, when there is only one Cys in the identified peptide. Oxidation of thiol groups to -SOH, has been proposed to be an intermediary step in the formation of disulfide bonds (Le Moan et al., 2006), although sulfenics are thought to be unstable there has been reports of stable long lived protein sulfenates (Le Moan et al., 2006). We have also used sodium arsenite to specifically reduce sulfenics (Saurin et al., 2004). Using glutaredoxin 2 yeast mutants we aim to detect, identify and compare proteins involved in disulfide bonds and containing SOH and help elucidate the mechanisms of cellular response to OS.

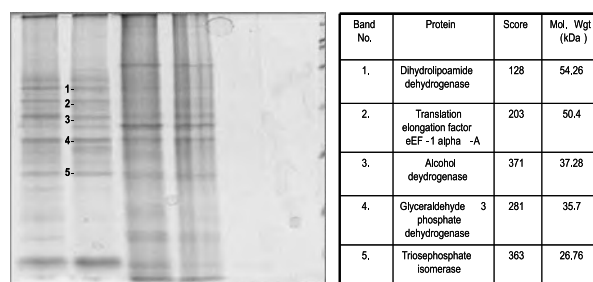


Figure 1. Lanes 1 & 2, proteins released from avidin beads, lanes 3 & 4 unbound proteins after incubation with avidin beads, lanes 5 & 6 absence of protein after final wash.

References

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Fosfoproteómica cuantitativa en células madre embrionarias humanas

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Las células madre embrionarias son un tipo celular proveniente de la masa interna del blastocisto que se caracterizan por su capacidad de autorenovación ilimitada y por ser pluripotentes ya que pueden originar cualquiera de los más de 200 tipos celulares que constituyen el ser humano. Debido a esta última propiedad, tienen un enorme potencial terapéutico sobre todo en el campo de la medicina regenerativa. En este sentido, se hace necesario profundizar en los mecanismos moleculares que gobiernan los procesos de diferenciación hacia un determinado destino celular, ya que esto permitirá, entre otras cosas, aumentar la eficiencia de los cultivos o mejorar la supervivencia de las células una vez transplantadas. Los mecanismos de señalización se transmiten, a menudo, mediante modificaciones post-traduccionales entre las que la fosforilación es una de las más importantes ya que controla numerosas funciones celulares. En nuestro estudio hemos utilizado una aproximación de proteómica cuantitativa para tra-

tar de definir los cambios a nivel de fosforilación implicados en las primeras etapas del proceso de diferenciación de células madre embrionarias hacia cardiomiocitos: 30, 60 y 240 minutos. Para ello nuestra aproximación combina SILAC para la cuantificación, SCX y TiO₂ para enriquecer en fosfopéptidos y espectrometría de masas (Orbitrap y FT-ICR) para la identificación. Así, hemos logrado identificar alrededor de 4000 fosfopéptidos, muchos de los cuales no han sido descritos hasta el momento. Más de 2000 han sido cuantificados, mostrando perfiles de expresión diferenciales en los tiempos estudiados. Nuestro trabajo muestra, por primera vez, los cambios dinámicos que tienen lugar en el fosfoproteoma de las células madre embrionarias humanas cuando se diferencian hacia cardiomiocitos y podría suponer un punto de partida en la optimización de las terapias celulares en pacientes que padezcan algún tipo de cardiomiopatía como por ejemplo el infarto de miocardio.