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A systematic approach to assess amino acid conversions in SILAC experiments

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ABSTRACT

SILAC is a widely accepted approach for quantitative proteomics in which proteins are labeled with stable isotopes during cell culture. A major drawback of this technique is the metabolic conversion of labeled amino acids that may hamper accurate quantification. A paradigmatic example of this phenomenon is the generation of labeled proline from arginine, known to occur in a good number of biological models. We propose a novel methodology to identify and quantitate metabolic conversions as well as to evaluate labeling efficiency in SILAC experiments. In this approach, labeled proteins are reduced to amino acids by acid hydrolysis before LC–MS/MS analysis. Since it is carried out at the amino acid level, tracking the fate of the isotope label is straightforward and can be performed for each amino acid independently. After applying this method to mammalian cells, grown in the presence of heavy arginine and lysine, labeling efficiency and amino acid conversions could be accurately evaluated. Only undesirable labeling of proline was found to occur at a significant extent, varying greatly among cell lines. Finally, increasing proline concentration in the growing medium was shown to be effective at preventing arginine conversion without any noticeable side effect.

1. Introduction

Stable Isotope Labeling with Amino acids in Cell culture (SILAC) is a broadly used approach in quantitative proteomics [1,2]. In this technique, cell populations to be compared are grown in medium containing either standard ("light") or stable isotope labeled ("heavy") amino acids. As a result, proteins synthesized in each condition are differentially labeled and, after mixing, quantitative differences between both states can be determined by mass spectrometry, without further modification of the proteomics workflow [3]. A number of amino acids have been employed in SILAC experiments so far, including leucine [3–5], isoleucine [6], methionine [7], tyrosine [5,7] and serine [5,7]. Nevertheless, arginine and lysine are typically the amino acids of choice [8,9] since trypsin cleaves after these residues. In this scenario, most peptides retain the label and are, thus, amenable for quantification.

Accurate quantification in SILAC experiments relies on two conditions: 1) complete incorporation of the labeled amino acid(s) and 2) absence of labeling in any other amino acid than the desired one(s). Although reliable quantitative information can be achieved from incomplete labeled samples [10], full incorporation of the heavy isotopes guarantees a broader dynamic range and is, by far, the ideal situation.

Regarding the second premise, it is known that, due to cellular metabolism, the isotope label may be transferred from one amino acid to another. A typical illustration of this phenomenon is the so-called arginine conversion problem, where degradation of this amino acid via the arginase pathway leads to the generation of labeled proline, biasing the quantitative ratios of proline containing peptides [11–14]. In some biological models, such as the fission yeast, amino acids other than proline have been reported to be affected by this artifact [15]. Several strategies have been proposed to either prevent arginine conversion [8,14,15] or to correct its detrimental effects [12,13,16]. Among the former, increasing proline concentration in the growing medium was reported to successfully avoid arginine conversion in human embryonic stem cells [14], although it has been suggested that such increase may lead to back conversion of proline into unlabeled arginine [1].

Characterization of amino acid conversions is usually performed at the peptide level [11–14]. However, since the isotope envelope of a peptide reflects the contribution of all its constituent residues, determination of isotope incorporation into other amino acids is not straightforward, especially if the conversion rate is low or if the peak clusters of the correctly and the undesirably labeled species overlap. In this paper, we propose a novel methodology to address

Abbreviations: BSA, bovine serum albumin; DMEM, Dulbecco's modified eagle's medium; EDTA, ethylenediaminetetraacetic acid; ESI, electrospray ionization; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; PBS, phosphate buffered saline; PFHA, perfluoroheptanoic acid; SILAC, Stable Isotope Labeling with Amino Acids in Cell culture.

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the amino acid conversion problem by performing the analysis of SILAC labeled samples at the amino acid level. Several human cell lines were labeled with lysine and arginine separately and their proteins were precipitated and subjected to acid hydrolysis. LC–MS/MS analysis of the amino acid mixture generated allowed accurate evaluation of labeling efficiency, and amino acid conversions could be analyzed systematically. Only arginine was found to metabolize to proline at a significant extent in the cell lines tested. Additionally, increasing proline concentration was effective at preventing undesirable labeling without any detectable back conversion into unlabeled arginine, or any other appreciable side effect. Thus, slight modifications in growing conditions make SILAC labeling with arginine and lysine a reliable approach for quantitative proteomics. Although the described protocol has been implemented for the evaluation of SILAC labeling in mammalian cells, any biological model, should be amenable to this kind of analysis.

2. Materials and methods

2.1. Cell lines and amino acids

SKHep (a kind gift of Dr. Iñaki Alvarez, Institut de Biotecnonologia i Biomedicina, Barcelona), HEK293 (a kind gift of Dr. Severine Gharbi, Centro Nacional de Biotecnología, Madrid) and HeLa cells were used. L-lysine and L-arginine as well as their labeled counterparts, $^{13}C_6{}^{15}N_2$-L-lysine and $^{13}C_6{}^{15}N_4$-L-arginine, were obtained from Silantes. L-proline was from Sigma–Aldrich.

2.2. Cell culture and SILAC labeling

DMEM medium without lysine and arginine (Silantes) was supplemented with 10% dialyzed fetal bovine serum (Gibco) and either 0.8 mM lysine plus 0.4 mM arginine (light condition), 0.8 mM $^{13}C_6{}^{15}N_2$-lysine plus 0.4 mM arginine (K8 condition), 0.8 mM lysine plus 0.4 mM $^{13}C_6{}^{15}N_4$-arginine (R10 condition) or 0.8 mM lysine, 0.4 mM $^{13}C_6{}^{15}N_4$-arginine plus proline 300 mg/l (R10 + P condition). Cells were cultured in parallel in the aforementioned conditions for seven days. After labeling, cells were washed twice with PBS and stored at −70 °C until further processing. Three biological replicates were carried out for each cell line in the four conditions.

2.3. Cell lysis, protein precipitation and acid hydrolysis

Cell pellets were resuspended in 200 μl of lysis buffer (1% Igepal CA-630, 10 mM Tris–HCl, 150 mM NaCl, 0.02% NaN_3, 1 mM EDTA) containing a mixture of protease inhibitors (Complete Mini without EDTA, Roche) and incubated at 4 °C for 30 min with gentle shaking. The lysate was centrifuged at 16,000 *g* for 10 min. The soluble fraction was transferred to a fresh tube and 4 volumes of methanol, 1 volume of chloroform and 3 volumes of water were added, vortexing vigorously after each addition. After centrifugation at 16,000 *g* for 2 min the aqueous top layer was removed and 4 volumes of methanol were added. The tube was vortexed and centrifuged at 16,000 *g* for 2 min. Liquid was removed and the protein pellet was left to air dry. Proteins were redissolved in water and quantified by Bradford assay using BSA as standard. 100 μg of protein were dried and resuspended in 100 μl of 6N HCl in a borosilicate tube under a stream of nitrogen. The tube was tightly sealed and incubated at 110 °C for 21 h. Afterwards, the tube was opened and incubated at 110 °C until complete evaporation. The solid residue was washed once with 100 μl of water and dissolved in 100 μl of 0.5 mM perfluorheptanoic acid (PFHA, Sigma–Aldrich) and filtered through a 0.22 μm Costar Spin-X filter tube (Sigma–Aldrich). The same procedure was applied for the acid hydrolysis of 100 μg of ovalbumin (Sigma–Aldrich).

2.4. Mass spectrometry analysis

25 μl (25 μg) of the amino acid mixture obtained after acid hydrolysis, was subjected to HPLC fractionation in an Ultimate system (Dionex) using an XBridge C18 column (3.5 μm, 2.1 × 50 mm, Waters) at a flowrate of 200 μl/min and 40 °C. Solvent A was 0.5 mM PFHA and solvent B was 100% acetonitrile. The gradient elution was conducted as follows: isocratic conditions with solvent A for 5 min, a linear increase to 10% solvent B in 1 min a linear increase to 50% solvent B in 14 min and isocratic conditions with 50% solvent B for 4 min.

The HPLC system was coupled online with a Squire 3000 ion trap (Bruker Daltonics) operating in positive mode via an electrospray ionsource. For each survey scan (25–300 m/z) the ion showing the most prominent signal was isolated for MS/MS fragmentation. An exclusion window of 0.5 min was applied after the acquisition of two fragmentation spectra of the same molecular species. Raw LC–MS data were processed using DataAnalysis 3.4 (Bruker Daltonics).

Individual MS/MS spectra for each amino acid were obtained by direct infusion of 10 μM of each of the 20 proteinogenic amino acids plus cystine (Sigma–Aldrich) in 80% acetonitrile, 0.1% formic acid.

3. Results and discussion

3.1. LC–MS analysis of underivatized amino acids

Estimation of labeling efficiency and detection of amino acid conversions are key points in any SILAC experiment. Labeling efficiency is usually evaluated at the peptide level by analyzing a subset of tryptic peptides [8,17] or by spectral counting of light versus heavy species [18]. Similarly, metabolic conversions of amino acids are indirectly inferred from the MS spectra of labeled peptides [12–15]. Since a tryptic digest is extremely complex compared with the mixture of its constituent amino acids we reasoned that the precision and accuracy of these estimations should increase if they were performed at the amino acid level. In particular, systematic evaluation of amino acid conversions should be much more straightforward and sensitive, given that each amino acid would be analyzed individually without interfering signals derived from the complex isotopic envelope of a peptide. To that end, we implemented an LC–MS/MS methodology to analyze amino acids pools obtained after acid hydrolysis of proteins.

Treatment with hydrochloric acid at 110 °C is a standard procedure to hydrolyze proteins into their constituent amino acids [19]. In these conditions, most amino acids are recovered quantitatively, exception made of tryptophan, which is degraded, and glutamine and asparagine that are converted to glutamic and aspartic acid, respectively [19]. In order to fractionate the mixture of underivatized amino acids, we took advantage of a previously described method based on the use of reversed-phase chromatography with PFHA acid as ion pairing agent [20,21]. Analysis of a hydrolysate of ovalbumin showed good chromatographic resolution and high reproducibility under our experimental conditions (Fig. 1 and Table 1). Identifications were performed by comparing the experimental MS2 spectra with those obtained upon direct infusion of standard solutions of each amino acid (Table 1).

As expected, no signals for tryptophan, glutamine or asparagine were observed. Additionally, cysteine was poorly detected while a prominent peak at minute 2.7 and m/z 241, subsequently assigned

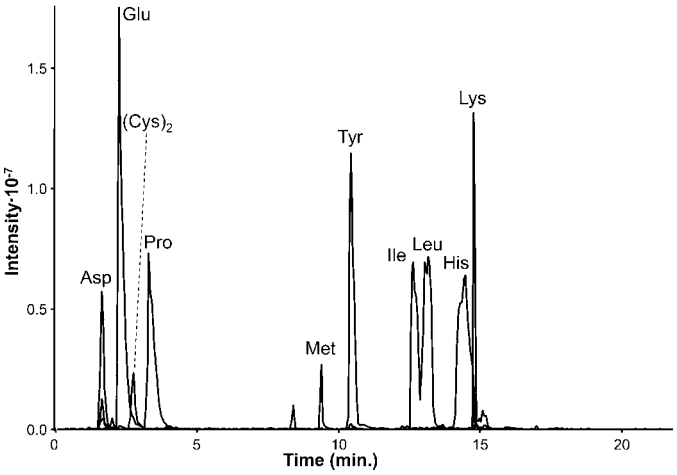

Fig. 1. Representative overlay of extracted ion chromatograms of several amino acids derived from the acid hydrolysis of ovalbumin. Amino acids are specified using the three letter code. $(Cys)_2$ represents cystine.

to cystine, was present (Fig. 1 and Table 1). In this regard, due to the highly acidic environment, oxidation of the sulfhydryl group of cysteine to form a disulfide bond, has been frequently reported during acid hydrolysis of proteins [19].

Although the amount of sample required for each LC–MS analysis (25 μg) is relatively high, the described method should be implemented without difficulty under nanoESI conditions. It is well known that flow rates in the nanoliter range may increase sensitivity more than two orders of magnitude [22], and that would allow to carry out the analysis with hundreds of nanograms or a few micrograms of sample. This would be especially advantageous when the amount of sample is limited, as may be the case in SILAC workflows. Furthermore, considering that a standard C18 column is employed for the fractionation of underivatized amino acids, the same instrumental setup that is routinely used for the MS analysis of tryptic peptides could be easily adapted to the methodology described, just by adding a new solvent line for 0.5 mM PFHA.

3.2. Evaluation of labeling efficiency

A potential source of error, often overlooked, in SILAC experiments is the isotope enrichment of commercial amino acids. To determine the purity of the $^{13}C_6{}^{15}N_2$-lysine and $^{13}C_6{}^{15}N_4$-arginine, 1 μg of each amino acid were analyzed by LC–MS as described above. Heavy lysine analysis revealed a major peak at m/z 155.13 corresponding to the $^{13}C_6{}^{15}N_2$ labeled amino acid (Fig. 2A). A second peak differing in 1 m/z unit from the major one (m/z 154.19) was present and accounted for about 7.5% of the total lysine signal. Similarly, in the case of arginine, a minor signal at m/z 184.15, representing about 5.3%, accompanied the one corresponding to the fully labeled arginine at m/z 185.1 (Fig. 2B). Both molecular species coeluted precisely, indicating that the lighter one corresponds to the amino acid lacking a ^{13}C or ^{15}N atom (Fig. 2). Additionally, no signal for the fully unlabeled amino acid could be detected either for arginine or lysine (at m/z 175.1 and 147.1, respectively).

SKHep, HEK293 and HeLa cells were labeled in four different conditions (see Section 2): Light lysine plus light arginine (light condition), heavy lysine plus light arginine (K8 condition), light lysine plus heavy arginine (R10 condition) and light lysine plus heavy arginine supplemented with an excess of unlabeled proline (R10 + P condition). To evaluate labeling efficiency, proteins from each condition were hydrolyzed and subjected to LC–MS analysis (Fig. 3). Labeling efficiency was similar and close to 100% in every case (Table 2). The fraction of light lysine or arginine after labeling was consistent with the expected one since, assuming a doubling time of 33 h, about 3.1% of unlabeled lysine or arginine should remain after 7 days of culture.

As expected, no differences in labeling efficiency were observed between lysine and arginine-labeled cells. This ensures that the

Table 2
SILAC labeling efficiency in HEK293, HeLa and SKHep cells. Mean ± SD of three biological replicates is shown.

	Lysine labeling efficiency (%)[a]	Arginine labeling efficiency (%)[b]	
	K8 condition	R10 condition	R10 + P condition
HEK293	96.0 ± 0.9	98.4 ± 0.5	97.7 ± 0.7
HeLa	96.4 ± 1.7	93.5 ± 4.4	96.1 ± 2.0
SKHep	98.5 ± 0.3	98.5 ± 0.3	98.1 ± 0.3

[a] Lysine labeling efficiency was estimated as the sum of areas of heavy lysine signals (m/z 154 and 155) relative to the sum of areas of light and heavy lysine signals (m/z 147, 154 and 155).
[b] Arginine labeling efficiency was estimated as the sum of areas of heavy arginine signals (m/z 184 and 185) relative to the sum of areas of light and heavy arginine signals (m/z 175, 184 and 185).

Table 1
Retention time, mass to charge ratio and major daughter ions in the MS2 spectrum of each of the amino acids detected after LC–MS/MS analysis of an acid hydrolysate of ovalbumin. Amino acids are specified using the three letter code. $(Cys)_2$ represents cystine.

Amino acid	Retention time (min)[a]	m/z (MS)	Daughter ions (MS2)[b]
Asp	1.7 ± 0.1	134	74, 88, 116
Ser	1.9 ± 0.1	106	60, 88
Gly	2.1 ± 0.1	76	30, 48
Glu	2.2 ± 0.1	148	84, 102, 130
Thr	2.3 ± 0.1	120	56, 74, 102
$(Cys)_2$	2.7 ± 0.2	241	120, 122, 137, 152, 154, 178, 195, 224, 225
Ala	2.8 ± 0.1	90	44
Pro	3.2 ± 0.1	116	70
Met	9.1 ± 0.3	150	56, 104, 133
Val	9.3 ± 0.1	118	72
Tyr	10.3 ± 0.2	182	136, 165
Ile	12.4 ± 0.3	132	86
Leu	12.8 ± 0.3	132	86
Phe	13.2 ± 0.2	166	120, 149
His	13.9 ± 0.6	156	95, 110
Lys	14.6 ± 0.3	147	84, 130
Arg	14.6 ± 0.3	175	60, 70, 116, 130, 157, 158

[a] Mean ± SD of four consecutive runs.
[b] The identity of each amino acid was confirmed by comparison of its MS2 spectrum with that obtained upon direct infusion of individual standard amino acids (see Section 2).

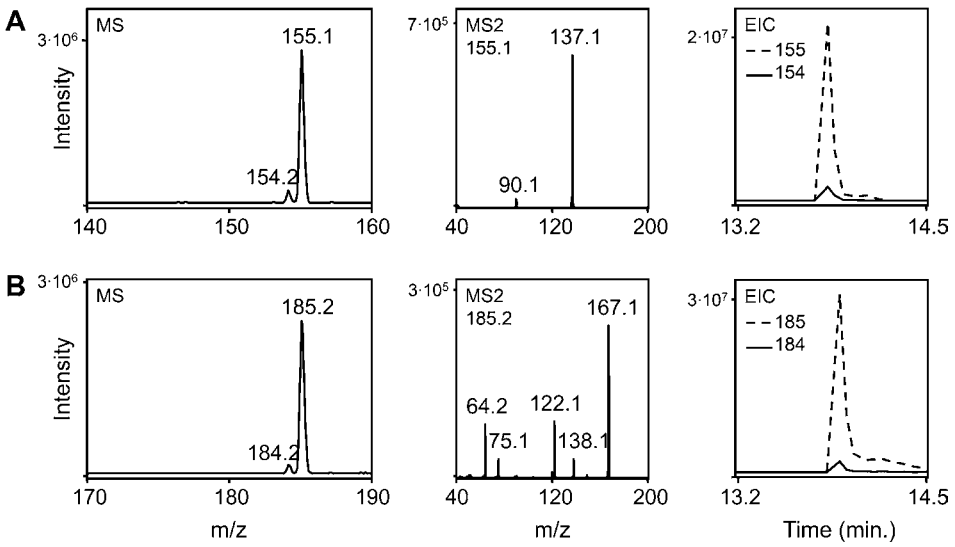

Fig. 2. MS spectrum (left panel), MS2 spectrum (middle panel) and extracted ion chromatogram (right panel) obtained after LC–MS/MS analysis of 1 μg of: (A) $^{13}C_6{}^{15}N_2$-lysine (*m/z* 155.1) and (B) $^{13}C_6{}^{15}N_4$-arginine (*m/z* 185.2).

bias derived from incomplete labeling is equivalent for lysine and arginine containing peptides, facilitating the normalization of quantitative ratios obtained from SILAC experiments.

3.3. Monitoring amino acid conversions during SILAC labeling

A major drawback of the SILAC approach derives from the fact that it is performed *in vivo*. Cell metabolism includes conversions of amino acids that, when affecting isotopically labeled species, may hamper accurate quantification. An illustrative example of this phenomenon is the generation of labeled proline from arginine (Fig. 4), known to occur in mammalian cells [12] as well as in yeast [11]. This causes the heavy signal of proline containing peptides to split in two, corresponding to the peptide containing light or heavy proline, while only the species containing light proline is taken into account for quantification. In addition, arginine conversion may affect other amino acids as it has been shown in *Schizosaccharomyces pombe*, where proline, glutamate, glutamine and lysine pools were partially labeled after arginine-based SILAC [15]. Additionally, changes in the culture medium may affect the extent of undesirable labeling [13,14]. Thus, it is advisable to properly assess amino acid conversions before performing SILAC in any biological

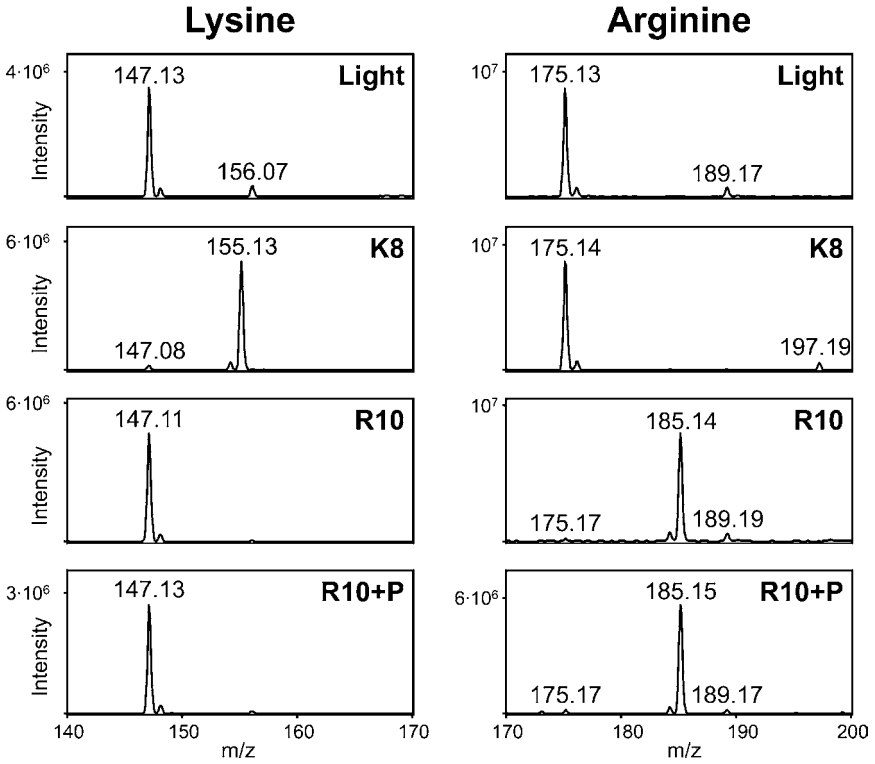

Fig. 3. Representative example of the MS spectra of lysine (left column) and arginine (right column) acquired after LC–MS/MS analysis of an acid hydrolysate of proteins from HEK293 cells grown in the light, K8, R10 or R10+P conditions. Signal at *m/z* 156 in the lysine spectra corresponds to histidine that partially coelutes with lysine (see Fig. 1 and Table 1). Signal at *m/z* 189, shifting to *m/z* 197 in the K8 condition, in the arginine spectra was identified as trimethyl-lysine (data not shown).

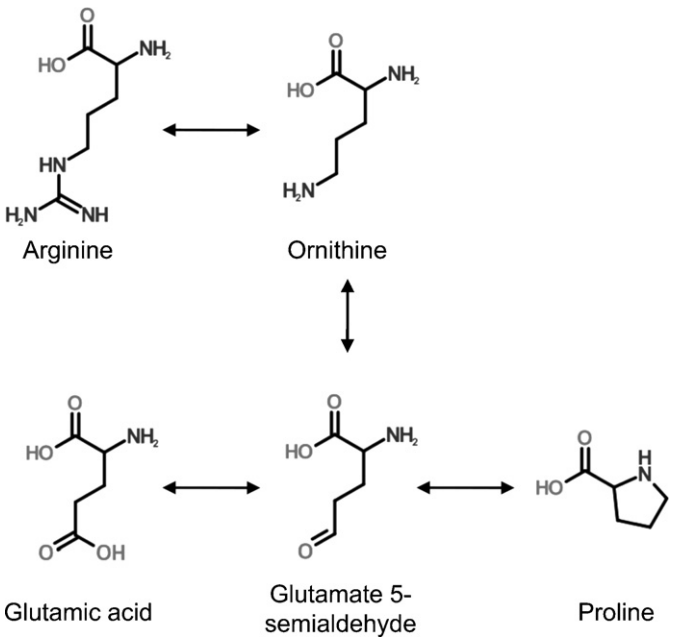

Fig. 4. Metabolic pathway involved in the conversion of arginine into proline. Modified from the KEEG pathway database (http://www.genome.jp/kegg/pathway.html).

model or when altering growing conditions. In this regard, monitoring isotope transfer at the amino acid level circumvents the difficulty of analyzing the complex isotopic envelopes of tryptic peptides when several residues are undesirably labeled [15] and allows the unequivocal determination of conversion rates regardless the amino acids involved or the mass shift introduced.

To investigate whether other, yet unidentified, metabolic conversions could occur in mammalian cells, we compared the MS spectra of each amino acid in the light, K8 and R10 conditions. We reasoned that, if heavy arginine and/or lysine were converted into another amino acid, its corresponding MS spectrum in the heavy condition should show an additional signal, not observed in the light one. The use of labeled amino acids with all their C and N atoms substituted with ^{13}C and ^{15}N, respectively, guaranteed that any conversion would be detected regardless of the region of the molecule involved. It might be argued that conversions involving only hydrogen/deuterium atoms would escape our detection. However, due to the different chromatographic behavior of deuterated amino acids [23] and the metabolically labile nature of the α-carbon bound hydrogen [24,25], there is growing consensus to avoid their use in SILAC. Thus, in this context, conversions involving exclusively hydrogen/deuterium atoms are of limited interest.

Three biological replicates for each cell line in the light, K8 and R10 conditions were analyzed and the MS spectrum of each amino acid was carefully inspected. Exception made of lysine, no differences were found between the MS spectra of the light and the K8 condition (data not shown) suggesting that conversion of lysine into other amino acids occurs to a negligible extent, if any, and that this amino acid is a suitable choice when performing SILAC labeling in mammalian cells.

Regarding the R10 condition, a signal at m/z 122.1, absent in the light condition, was detected (Fig. 5A) showing the same retention time as proline (m/z 116.1). The precise coelution of both species and their fragmentation pattern (data not shown) were consistent with the assumption that the ion at m/z 122 corresponds to $^{13}C_5{}^{15}N$-proline that would derive from the metabolic conversion of $^{13}C_6{}^{15}N_4$-arginine. This finding validates our approach for the detection of undesirable labeling of amino acids in SILAC experiments.

The extent of arginine conversion varied greatly among cell lines, ranging from $1.4 \pm 0.1\%$ in HeLa to $13.5 \pm 3.4\%$ in HEK293 cells (Fig. 5B). Moreover, conversion efficiencies close to 40% have been previously reported in mammalian cells [13]. This stresses the importance of accurate determination of the rate of artifactual labeling of proline for each experiment individually, in order to achieve reliable quantitative information.

No other amino acid was found to be enriched in ^{15}N or ^{13}C in the R10 condition (data not shown), indicating that generation of labeled proline from arginine is the only metabolic conversion that may alter quantification ratios in arginine-based SILAC experiments in the cell lines tested.

A potential criticism to this approach is that, since tryptophan, glutamine and asparagine are lost during acid hydrolysis, our analysis is blind to changes affecting those amino acids. Yet, glutamine and asparagine are recovered as their corresponding carboxylic acids (glutamic and aspartic acid, respectively) and, given that no alterations were detected in the later, it is unlikely that any metabolic conversion could affect the former. Regarding tryptophan, as its frequency in proteins is rather low, about 1.25% in the human proteome according to the integr8 database (http://www.ebi.ac.uk/integr8), the risk of error due to undesired labeling of this amino acid is scarce. Nevertheless, simple modifications in the acid hydrolysis protocol have been described to allow the recovery and analysis of tryptophan if needed [19,26].

3.4. Prevention of arginine to proline conversion

Several ways of dealing with the arginine to proline conversion problem have been suggested so far. For simplicity, they can be divided into the following categories: 1) methods that correct the quantitative ratios of proline containing peptides during data analysis but do not avoid arginine conversion, 2) methods that use two different isotopomers of labeled arginine in the light and heavy conditions so that labeling of proline occurs at the same rate in both states and 3) methods that effectively prevent arginine conversion.

Correction of labeling ratios during data analysis is usually performed by adding up the contribution of the light and heavy proline peaks [12,16]. This approach, however, not only complicates the analysis but increases the chances of coelution events that are known to hinder proper quantification [8,27]. As an alternative, Van Hoof et al. envisaged an approach where $^{15}N_4$- and $^{15}N_4{}^{13}C_6$-arginine were used in the light and heavy conditions, respectively [13]. Arginine conversion in the light state yields ^{15}N-labeled proline instead of light one and accurate quantification ratios can be achieved by comparison of the intensities of the monoisotopic peak of the light and heavy species. One disadvantage of this method is that quantification is carried out solely on the basis of monoisotopic peaks, which may affect accuracy [16]. In addition, the use of two distinct labeled isotopomers of arginine increases the cost of each SILAC experiment considerably.

The last set of methods aims at preventing arginine conversion by either modifying the cells genetically or altering the composition of the growth medium. Genetic engineering has been successfully applied in *S. pombe* to delete genes involved in arginine conversion to other amino acids [15]. Although it may be a practical approach in yeast, especially when undesirable labeling affects several amino acids, its application in higher eukaryotes seems unfeasible. In this regard, prevention of arginine conversion in mammalian cells can be accomplished by either reducing the amount of labeled arginine [8] or increasing proline concentration [14] in the medium.

Suboptimal arginine concentration has been shown to affect growth in a good number of cell lines [13,28] and may even result in the incorporation of unlabeled arginine, probably derived from

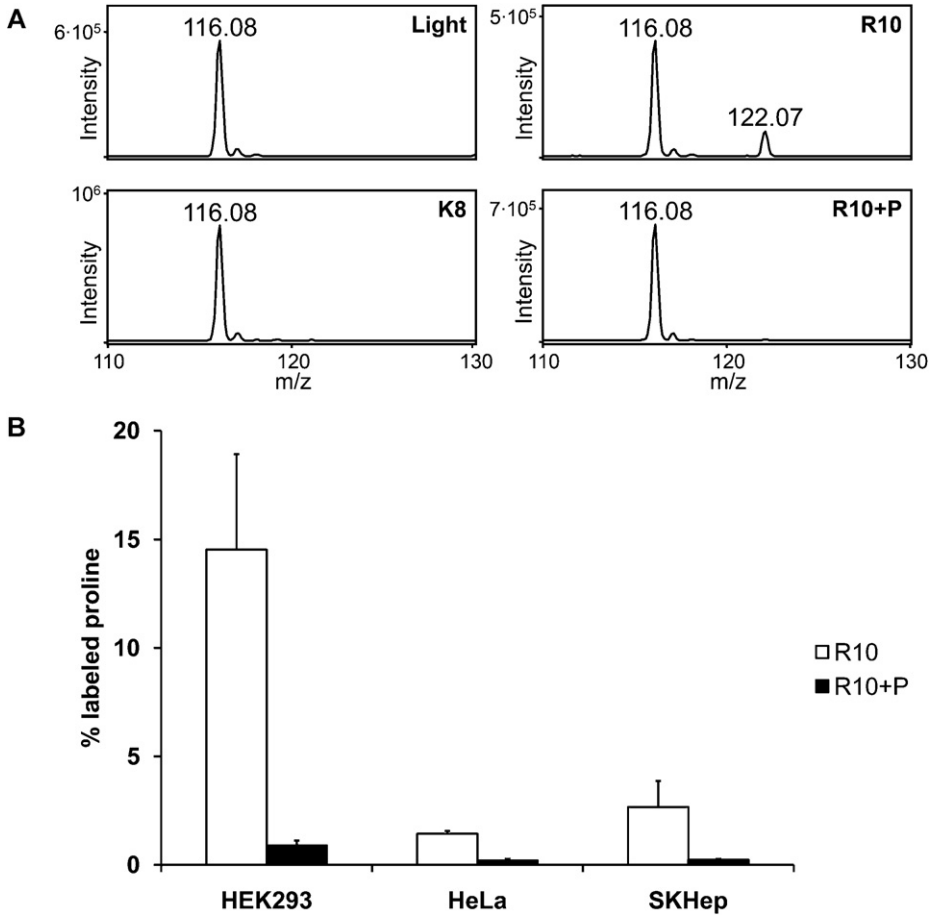

Fig. 5. Evaluation of the efficiency of arginine to proline conversion in HEK293, HeLa and SKHep cells. (A) Representative example of the MS spectra of proline (m/z 116) from HEK293 cells in the light, K8, R10 and R10 + P conditions. Note the signal at m/z 122 in the R10 condition corresponding to $^{13}C_5{}^{15}N$-proline. (B) Efficiency of proline labeling for the three cell lines in the R10 (white bars) and R10 + P (black bars) conditions. Mean ± SD of three biological replicates is shown for each cell line.

serum proteins [13], limiting the potential targets of this approach. On the other hand, it has been suggested that an excess of unlabeled proline could lead to its back conversion into unlabeled arginine [1]. Furthermore, since glumatamate 5-semialdehyde is an intermediate in the synthesis of both proline and glutamate from arginine (Fig. 5), supplementing the cells with the former might lead to isotope labeling of the later.

To evaluate the effectiveness of proline addition in preventing undesirable isotope incorporation we compared the MS spectra of amino acids deriving from the Light, R10 and R10 + P conditions. Arginine conversion was almost abolished in the presence of 300 mg/l of proline (Fig. 5). Even in the HEK293 cell line, which showed the highest rate of arginine conversion, a more than ten-fold reduction could be achieved. Interestingly, labeling efficiency was undistinguishable in the light, R10 or R10 + P conditions (Fig. 3 and Table 2), indicating that back conversion into arginine does not occur or is negligible. This is in agreement with the fact that arginine is essential for most cells in culture [28] and, thus, cannot be synthesized from other amino acids at a significant extent. Furthermore, no labeling of glutamic acid or any other amino acid except arginine was detected. On the whole, these data indicate that addition of unlabeled proline is a simple, yet effective, way of avoiding arginine conversion with no apparent side effects in SILAC experiments.

4. Conclusions

Labeling efficiency and metabolic conversion of amino acids are critical issues that should be tightly controlled in any SILAC experiment. We propose a fast and straightforward approach to address both questions. Our strategy makes use of acid hydrolysis to reduce a complex sample of proteins to a much simpler mixture of amino acids that is then analyzed by LC–MS. Since the analysis is carried out at the amino acid level, the fate of the label in any conversion event can be tracked systematically and its incorporation into any amino acid can be readily assessed.

After applying this method to several mammalian cell lines, isotopically labeled with arginine and lysine, it was concluded that: 1) evaluation of labeling efficiency and amino acid conversions can be accurately performed at the amino acid level, 2) labeled lysine is not converted into any amino acid, 3) only arginine to proline conversion occurs at a significant extent and 4) increasing proline concentration can effectively prevent this metabolic conversion without back conversion into unlabeled arginine, or any other appreciable side effect.

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