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ARTICLE

Hydrogen exchange during cell-free incorporation of deuterated amino acids and an approach to its inhibition

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Abstract Perdeuteration, selective deuteration, and stereo array isotope labeling (SAIL) are valuable strategies for NMR studies of larger proteins and membrane proteins. To minimize scrambling of the label, it is best to use cell-free methods to prepare selectively labeled proteins. However, when proteins are prepared from deuterated amino acids by cell-free translation in H_2O, exchange reactions can lead to contamination of ^2H sites by ^1H from the solvent. Examination of a sample of SAIL-chlorella ubiquitin prepared by *Escherichia coli* cell-free synthesis revealed that exchange had occurred at several residues (mainly at Gly, Ala, Asp, Asn, Glu, and Gln). We present results from a study aimed at identifying the exchanging sites and level of exchange and at testing a strategy for minimizing ^1H contamination during wheat germ cell-free translation of proteins produced from deuterated amino acids by adding known inhibitors of transaminases (1 mM aminooxyacetic acid) and glutamate synthetase (0.1 mM L-methionine sulfoximine). By using a wheat germ cell-free expression system, we produced [U–^2H, ^{15}N]-chlorella ubiquitin without and with added inhibitors, and [U–^{15}N]-chlorella ubiquitin as a reference to determine the extent of deuterium incorporation. We also prepared a sample of [U–^{13}C, ^{15}N]-chlorella ubiquitin, for use in assigning the sites of exchange. The added inhibitors did not reduce the protein yield and were successful in blocking hydrogen exchange at C^{α} sites, with the exception of Gly, and at C^{β} sites of Ala. We discovered, in addition, that partial exchange occurred with or without the inhibitors at certain side-chain methyl and methylene groups: Asn–H^{β}, Asp–H^{β}, Gln–H^{γ}, Glu–H^{γ}, and Lys–H^{ε}. The side-chain labeling pattern, in particular the mixed chiral labeling resulting from partial exchange at certain sites, should be of interest in studies of large proteins, protein complexes, and membrane proteins.

Keywords Cell-free translation · Chlorella ubiquitin · SAIL · Perdeuterated protein · Proton back exchange · Transamination · Transaminase inhibitor

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Introduction

Perdeuteration, selective deuteration, and stereo array isotope labeling (SAIL) are powerful strategies used in studying the structure and dynamics of large proteins, membrane proteins and protein–protein complexes by NMR spectroscopy (Kainosho and Güntert 2009; Tugarinov et al. 2006). SAIL amino acids are synthesized to contain an optimal labeling pattern that minimizes spin diffusion and allows for

stereospecific assignment of prochiral groups (Kainosho et al. 2006). The incorporation of a full set of SAIL amino acids or other selectively labeled amino acids requires that proteins be prepared by cell-free synthesis so as to minimize scrambling of the label (Klammt et al. 2007). However, it has been noted that deuterated amino acids introduced into proteins by *E. coli* cell-free synthesis can undergo exchange with ^{1}H from the solvent during the reaction so as to introduce ^{1}H at C^{α} and C^{β} positions (Etezady-Esfarjani et al. 2007).

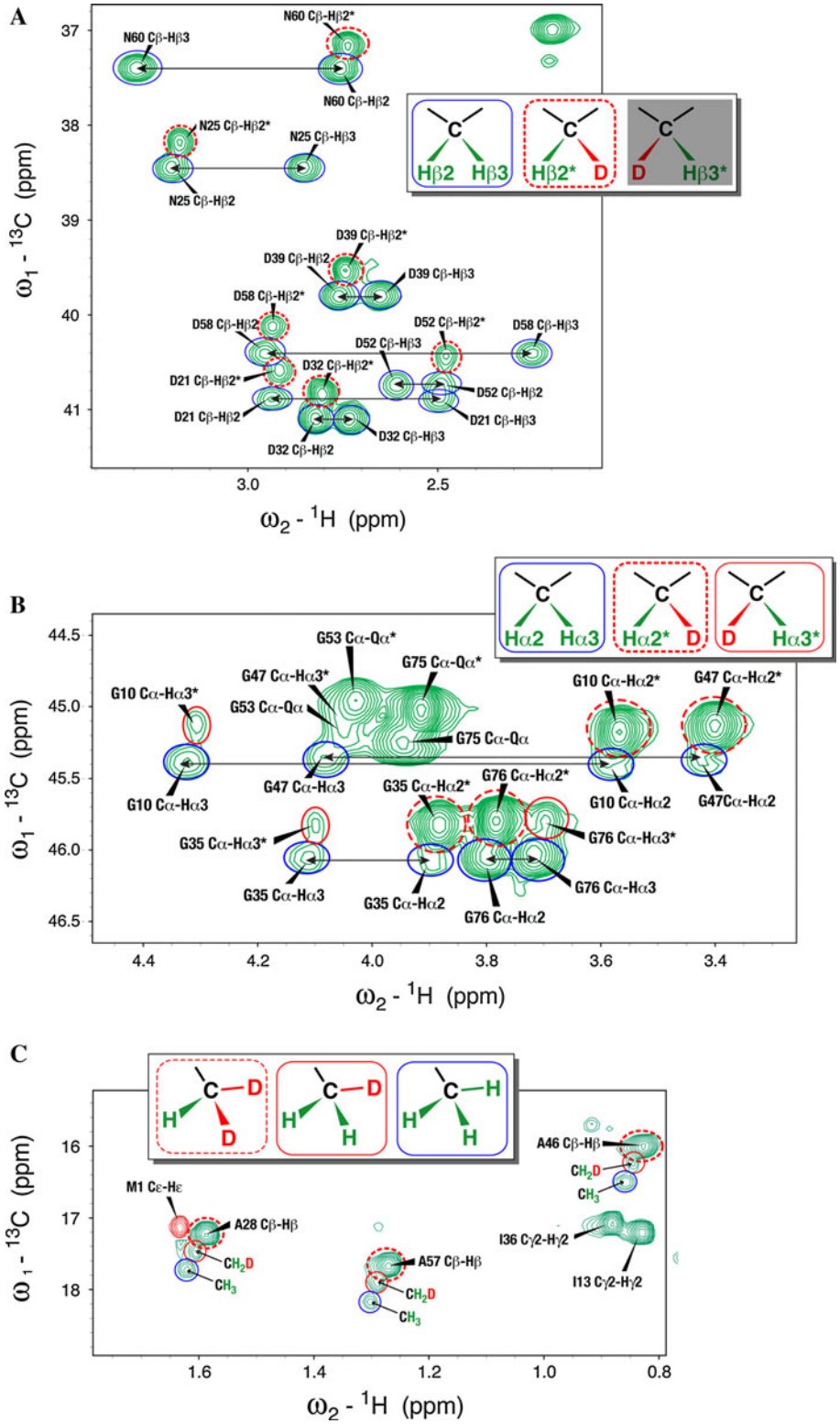


Fig. 1 Expansions of the 2D $^{1}H,^{13}C$–HSQC spectrum of SAIL chlorella ubiquitin prepared by *E. coli* cell-free protein synthesis. **A** $^{13}C^{\beta}$–$^{1}H^{\beta}$ region for Asp and Asn residues. The peak corresponding to the original $^{13}C^{\beta}$–($H^{\beta 2}$,$D^{\beta 3}$) group is indicated by a *dashed red circle* and labeled with an *asterisk*. Back exchange gave rise to two peaks corresponding to the non-degenerate CH_2 protons that are *circled* in *blue* and connected by *arrows*. **B** Gly $^{13}C^{\alpha}$–$^{1}H^{\alpha}$ region. Four peaks were observed for each Gly residue: a major peak from the original $^{13}C^{\alpha}$–($H^{\alpha 2}$,$D^{\alpha 3}$) indicated by a *dashed red circle* and an *asterisk*, a minor peak from a species resulting from internal exchange $^{13}C^{\alpha}$–($D^{\alpha 2}$,$H^{\alpha 3}$) indicated by a *red circle* and an *asterisk*, and a doublet from $^{13}C^{\alpha}$–($H^{\alpha 2}$,$H^{\alpha 3}$) indicated by *blue circles* connected by *arrows*. **C** Alanine methyl region peaks showing signals from the original –CHD_2 (*dashed red circle*) species and–CH_2D (red circle) and –CH_3 species (*blue circle*) resulting from exchange. The inset in each *panel* indicates the species that are observed for each group. The *circles* surrounding each group in the inset match the *circles* around the peaks. The species not observed for Asn/Asp, $^{13}C^{\beta}$–($D^{\beta 2}$,$H^{\beta 3}$), is *grayed* out

We report here evidence for such exchange reactions at backbone and side chain positions with SAIL amino acids incorporated into chlorella ubiquitin prepared by *E. coli* cell-free synthesis and undertook to determine which amino acids and which positions were affected. We then observed a similar exchange pattern in a sample of per-deuterated chlorella ubiquitin prepared in a wheat germ cell-free protein production system. In the same system, we also found that most, but not all, of these exchange reactions could be inhibited by the addition of inhibitors of transaminases (1 mM aminooxyacetic acid) and glutamine synthetase (0.1 mM L-methionine sulfoximine).

Experimental section

Materials

[^{15}N]-, [U–^{13}C, ^{15}N]-, [U–^{2}H, ^{15}N]-labeled mixtures of 20 amino acid were obtained from Cambridge Isotope Laboratories. Wheat germ extract was purchased from CellFree Sciences. Aminooxyacetic acid and L-methionine sulfoximine were purchased from Sigma-Aldrich. The SAIL amino acids were from SAIL Technologies, Inc.; the deuterium labeling pattern was that published (Kainosho et al. 2006) with the following differences: Ser 3S, Gly 2S, Pro 5R and His 3R.

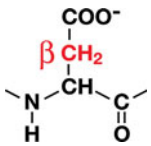
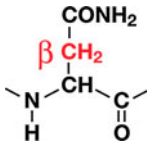
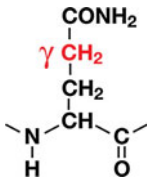
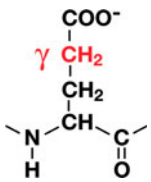
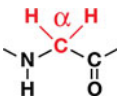
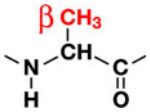
Cell-free protein preparation

SAIL-chlorella ubiquitin was prepared by *E. coli* cell-free synthesis with S30 extract from *E. coli* BL-21 (DE3) as described previously (Ikeya et al. 2009). The cell-free transcription/translation reaction was carried out at 37°C over a period of 7 h. For wheat germ cell-free protein production, the open reading frame coding for the protein was inserted into a pEU vector that codes for an N-terminal His_6 fusion with a TEV protease cleavage site. Samples of chlorella ubiquitin were produced by wheat germ cell-free translation at the Center for Eukaryotic Structural Genomics (CESG) by methods described previously (Makino et al. 2010; Vinarov et al. 2006). Cell-free synthesis was carried out in a Protemist100 (CellFree Sciences) as six 4-ml reactions each in the presence of a mixture of 20 labeled amino acids at 0.1% (w/v). Cell-free translations were carried out at 26°C over a period of 18 h. Reactions were carried out in the presence of inhibitors added to the cell-free reaction mixture (1 mM aminoxyacetate and 0.1 mM L-methionine sulfoximine) and appropriately labeled mixtures of amino acids to prepare samples with three labeling patterns: [U–^{15}N]-, [U–^{2}H, ^{15}N]-, and [U–^{15}N, ^{13}C]-chlorella ubiquitin. A second sample of [U–^{2}H, ^{15}N]-chlorella ubiquitin was prepared in the absence of inhibitors. Each labeled protein was isolated by Ni–NTA affinity chromatography, and the tag was cleaved by His-tagged TEV protease. Next, subtractive Ni–NTA affinity chromatography was used to remove the uncleaved protein, the cleaved tag, and the TEV protease. Finally, the chlorella ubiquitin sample was purified size exclusion chromatography and concentrated for NMR analysis.

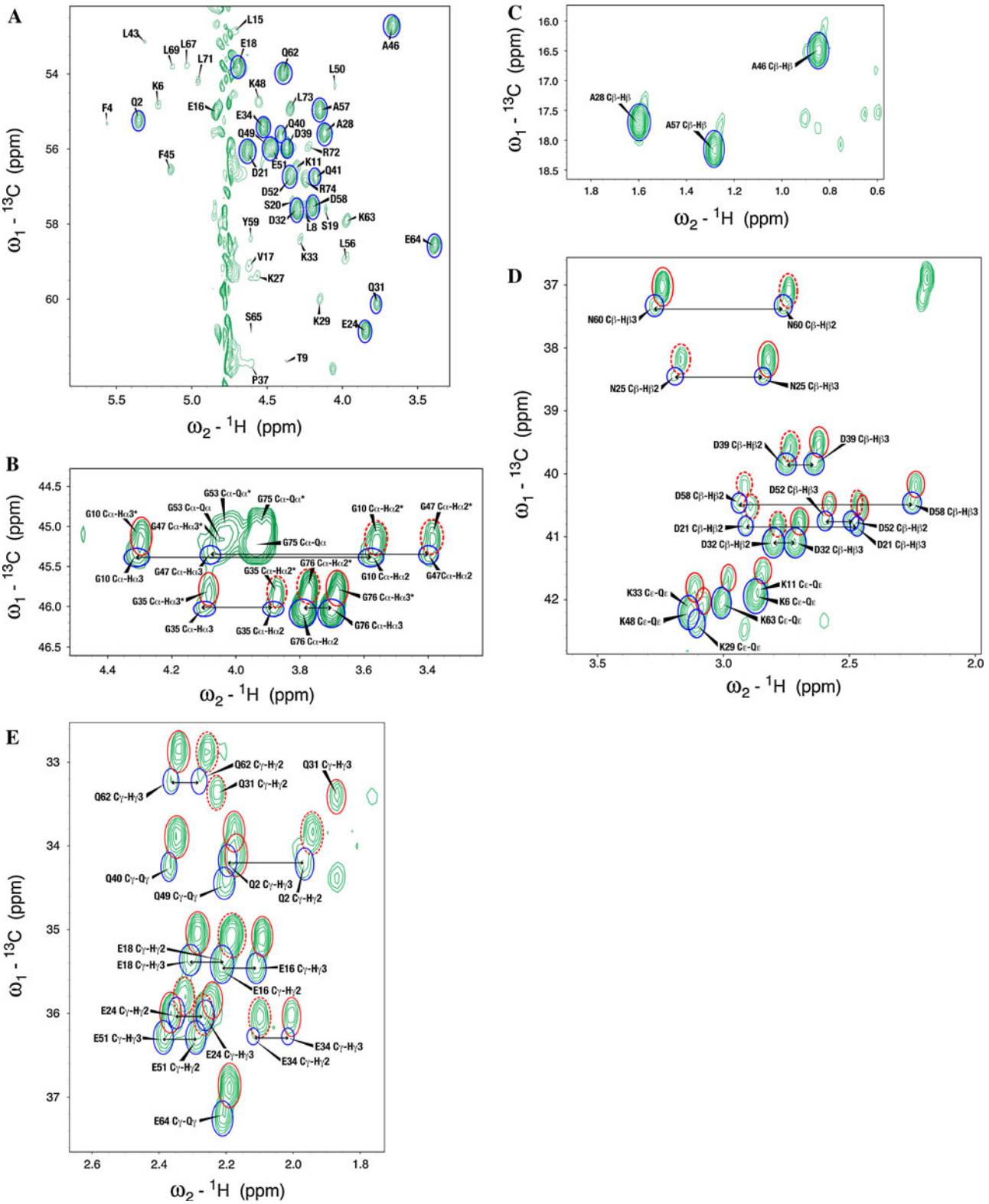
NMR spectroscopy

All NMR spectra were recorded at the National Magnetic Resonance Facility at Madison (NMRFAM) on 800 and

Table 1 Relative populations of protonated species in SAIL ubiquitin

Methylene groups			CHD	CH_2	CDH
Asp		D21/D32 D39/D52 D58	45% ±3	55% ±3	
Asn		N25/N60	47% ±1	53% ±1	
Gln		Q40/Q49	91% ±1	9% ±1	
Glu		E34/E64	76%	9%	15%
Gly		G76/G10	74% ±4	19% ±5	7% ±1
Methyl groups			CHD_2	CH_2D	CH_3
Ala		A28/A46 A57	81% ±1	10% ±1	9% ±1

Data are shown only for groups exhibiting significant exchange that could be easily integrated. The β-methylene groups of Asp and Asn show more than 50% D → H back-exchange, resulting in the appearance of three distinct 2D ^{1}H,^{13}C–HSQC peaks with similar intensity for each methylene group (Fig. 1A). Columns headed by CHD and CHD_2 give the relative protonation at the SAIL prochiral position. Volumes were measured with SPARKY software by fitting the shape of the peak to a Gaussian model



600 MHz Varian VNMRS spectrometers each equipped with a triple-resonance cryogenic probe. The temperature of each sample was regulated at 25°C. Sequence-specific assignments were determined for SAIL-chlorella ubiquitin and for [U–^{13}C,^{15}N]-chlorella ubiquitin. A series of 2D and 3D heteronuclear NMR spectra were collected on a sample of 1.1 mM [U–^{13}C,^{15}N]-chlorella-ubiquitin dissolved in NMR buffer containing 10 mM phosphate, 0.04% NaN_3,

◀ **Fig. 2** Expansions of the 2D ^{1}H,^{13}C–HSQC spectrum of [U–^{2}H, ^{15}N] chlorella ubiquitin prepared by wheat germ cell-free protein expression. **A** $^{13}C^{\alpha}$–H^{α} region for all residues but Gly. Peaks from the residue types showing the most extensive exchange (Ala, Asp, Glu, and Gln) are *circled* in *blue*. **B** $^{13}C^{\alpha}$–H^{α} region for Gly residues. Peaks resulting from back exchange are circled *red* for one methylene proton and *blue* for two methylene protons; *arrows* connect peaks from non-degenerate CH_2 protons; *asterisks* indicate peaks resulting from CHD or CDH groups. *Dashed red* circles identify signals matching the prochiral SAIL labeling pattern. Signals from residues with degenerate $^{1}H^{\alpha}$ chemical shifts are labeled 'Cα–Qα'. **C** Ala methyl region showing D → H exchange mostly to $–CH_3$ species (*circled* in *blue*). (**D**) $^{13}C^{\beta}$–$^{1}H^{\beta}$ region of Asp and Asn residues and $^{13}C^{\varepsilon}$–$^{1}H^{\varepsilon}$ region of Lys residues. Peaks resulting from back exchange are *circled red* for one methylene proton and *blue* for two methylene protons; *arrows* connect peaks from non-degenerate CH_2 protons. *Dashed red circles* identify signals matching the prochiral SAIL labeling pattern. Degenerate signals are labeled 'Cε–Qε'. (**E**) $^{13}C^{\gamma}$–$^{1}H^{\gamma}$ region of Glu and Gln residues. Peaks resulting from back exchange are circled *red* for one methylene proton and *blue* for two methylene protons; *arrows* connect peaks from non-degenerate CH_2 protons. *Dashed red circles* identify signals matching the prochiral SAIL labeling pattern. Degenerate signals are labeled 'Cγ–Qγ'

90% H_2O and 10% D_2O at pH 6.6. Raw NMR data were processed with NMRPipe (Delaglio et al. 1995) and analyzed using the programs XEASY (Bartels et al. 1995) and SPARKY (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco). 2D ^{1}H–^{15}N HSQC and 3D HNCO data sets were used to identify the number of spin systems, and these identifications plus 3D HNCACB and 3D CBCA(CO)NH data sets were used as input to the PINE server (Bahrami et al. 2009) to determine sequence specific backbone resonance assignments. Backbone resonance assignments were confirmed on the basis of ^{15}N-resolved ^{1}H–^{1}H NOESY data. 2D ^{1}H–^{13}C HSQC, 3D HBHA(CO)NH, 3D HC(CO)NH, 3D C(CO)NH experiments were used to assign the side chain and $^{1}H^{\alpha}$ and $^{1}H^{\beta}$ resonances. A series of 2D ^{1}H–^{15}N HSQC, 2D ^{1}H–^{13}C HSQC (natural abundance), 3D ^{15}N resolved ^{1}H–^{1}H NOESY experiments were recorded with identical parameters and measurement times for samples containing 0.8 mM [U–^{15}N] protein and 0.7 mM [U–^{2}H,^{15}N] protein produced with and without the inhibitors. Proton back exchange sites were identified and quantified through analysis of 2D ^{1}H–^{13}C–HSQC spectra acquired at natural abundance ^{13}C.

Results and discussion

Proton back exchange in SAIL ubiquitin

Standard triple resonance experiments were used in determining sequence-specific backbone and side chain assignments for the sample of SAIL-chlorella-ubiquitin prepared by *E. coli* cell-free expression. The 2D ^{1}H–^{13}C–HSQC spectrum of SAIL-chlorella-ubiquitin showed evidence for proton back exchange at several sites: H^{α} of Gly; H^{β} of Ala, Asn, and Asp; and, to a lesser extent, H^{γ} of Glu and Gln residues. Instead of the expected single $^{1}H^{\alpha}$ peak from prochiral ^{2}H labeled Gly, each Gly residue yielded four cross peaks: a major peak from the original $^{13}C^{\alpha}$–($H^{\alpha 2}$,$D^{\alpha 3}$), a minor peak from a species resulting from internal exchange $^{13}C^{\alpha}$–($D^{\alpha 2}$,$H^{\alpha 3}$), and a doublet from $^{13}C^{\alpha}$–($H^{\alpha 2}$,$H^{\alpha 3}$) (Fig. 1B). The $^{13}C^{\alpha}$ chemical shifts of the first two species were very similar, but that of the third was shifted by 0.3 ppm because of the deuterium isotope effect. Similarly, three signals were observed for the β-methylene groups of Asn and Asp residues. One resonance corresponds to the chiral $^{13}C^{\beta 1}$–($H^{\beta 2}$,$D^{\beta 3}$) group from the SAIL labeling, while the other two peaks at a common ^{13}C position result from the non-degenerate proton signals of the $^{13}C^{\beta 1}$–($H^{\beta 2}$,$H^{\beta 3}$) group (Fig. 1A). In addition, the Ala residues showed evidence of D → H exchange resulting in three distinct signals with slightly different proton and carbon frequencies and different percent occupancy for the three protons of the Ala β-methyl. One signal corresponds to the chiral CD_2H SAIL labeled species, while the other two arise from the CH_2D and CH_3 groups (Fig. 1C). The relative levels of protonated species were calculated for back-exchanged groups with isolated peaks that could be reliably integrated (Table 1). Notably, more than 50% of the D in the β-positions of Asp and Asn showed exchange to H, resulting in the appearance of three peaks of similar intensity (Fig. 1A). Such observations might not have been reported for previous SAIL labeled proteins, because, given the larger size of the proteins being studied, the additional peaks were either not seen due to line broadening or were overlapped by peaks from other groups.

Proton back exchange in perdeuterated ubiquitin

To better understand the mechanism of proton back exchange observed in SAIL ubiquitin and to evaluate strategies for the preparation of perdeuterated proteins by wheat germ cell-free translation, we prepared a sample of ubiquitin by wheat germ cell-free translation with [U–^{2}H,^{15}N]-labeled amino acids. Significant back exchange was observed at the α-positions of Gly, Ala, Asp, Glu, and Gln residues as shown in the $^{13}C^{\alpha}$–$^{1}H^{\alpha}$ region expansion of a 2D ^{1}H–^{13}C–HSQC spectrum in Fig. 2A and B. Proton back exchange was also observed at the β-positions of Ala, Asp, and Asn residues (Fig. 2C and D). In addition, proton back exchange was observed at the γ-positions of Glu, Gln (Fig. 2E), and the ε-positions of Lys residues (Fig. 2D).

Presumably exchange at α-positions is catalyzed by transaminases (Oshima and Tamiya 1961, Su et al. 2011). To

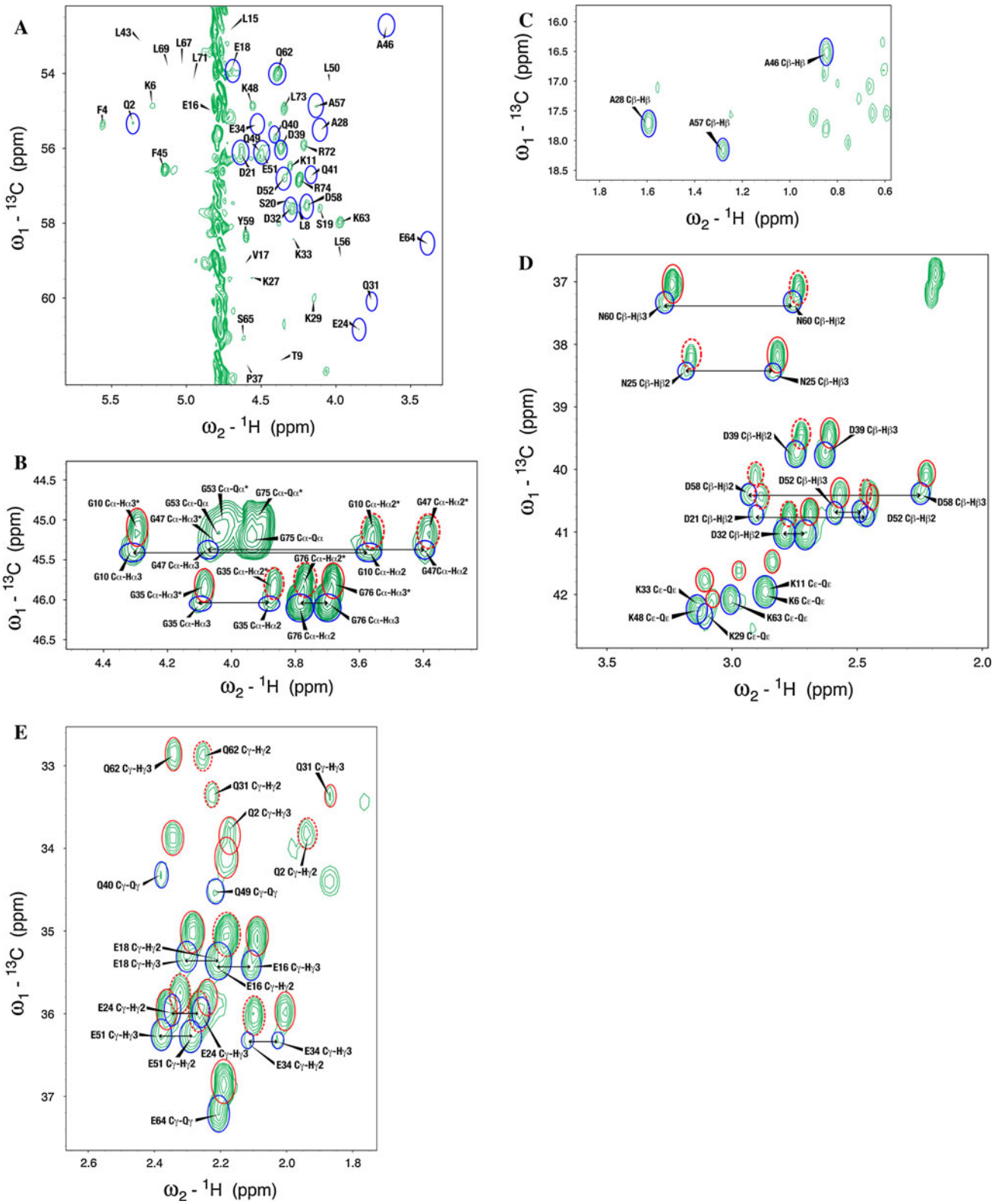


Fig. 3 Expansions of the 2D ^{1}H,^{13}C–HSQC spectrum of [U–^{2}H, ^{15}N] chlorella ubiquitin prepared by wheat germ cell-free protein expression in presence the transaminase inhibitors. Labeling is as in Fig. 2 **A** $^{13}C^{\alpha}$–$^{1}H^{\alpha}$ region for all residues but Gly. **B** $^{13}C^{\alpha}$–$^{1}H^{\alpha}$ region for Gly residues. **C** Ala methyl region. **D** $^{13}C^{\beta}$–$^{1}H^{\beta}$ region of Asp and Asn residues and $^{13}C^{\varepsilon}$–$^{1}H^{\varepsilon}$ region of Lys residues. **E** $^{13}C^{\gamma}$–$^{1}H^{\gamma}$ region of Glu and Gln residues. To allow comparison of peak intensities, all the spectra were plotted using parameters identical to those used for Fig. 2

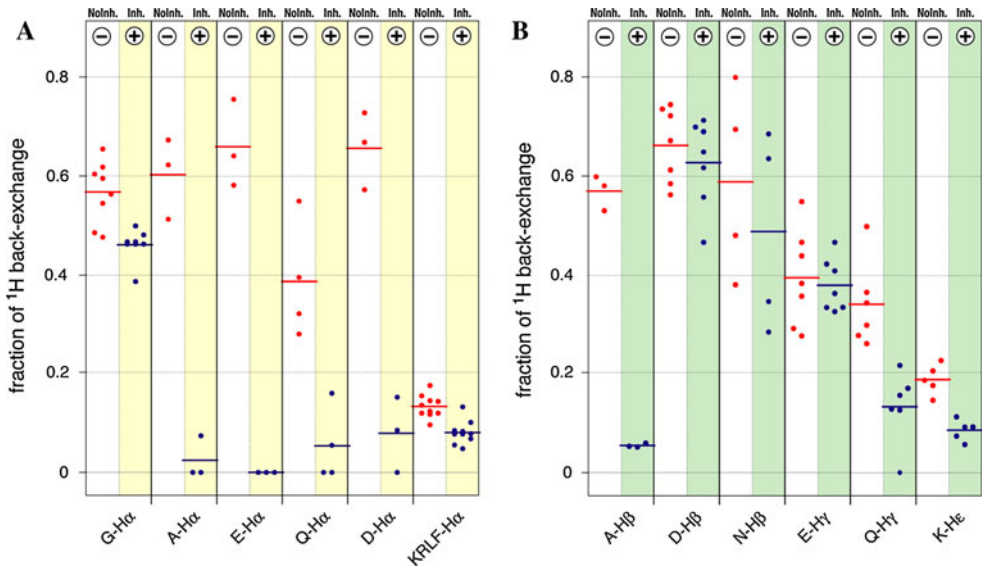


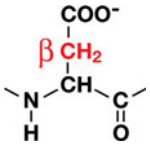
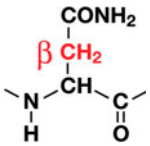
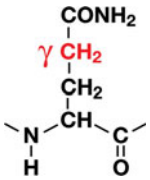
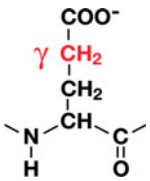
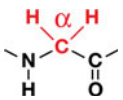
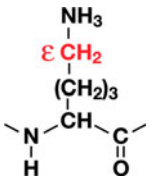
Fig. 4 Extent (fraction) of D → H back exchange at different residue types of chlorella ubiquitin that occurred during the wheat germ cell-free translation reaction in H_2O with a mixture of [U–^{2}H, ^{15}N]-labeled amino acids in the absence (*red*) and presence (*blue*) of the inhibitors. Values at individual residues are depicted by *dots* and averages by *horizontal bars*. **A** Exchange at backbone α-positions. **B** Exchange at side chain positions as indicated. The fraction of back-exchange is calculated such that the sum of all protonated and deuterated species adds up to 1. The contribution from deuterated species (invisible in the spectra) was estimated by comparing the volume of each peak in the 2D ^{1}H,^{13}C–HSQC spectrum of [U–^{2}H, ^{15}N]-ubiquitin with the volume of the corresponding peak in the 2D ^{1}H,^{13}C–HSQC spectrum of a fully protonated sample of [U–^{15}N]-ubiquitin. The volumes of the 2D ^{1}H,^{13}C–HSQC peaks from the different ubiquitin samples were normalized based on 2D ^{1}H,^{15}N–HSQC spectra acquired for all ubiquitin samples back-to-back on the same spectrometer using the same acquisition parameters and processed identically. Table 2 contains a more detailed analysis of the distribution of the various protonated species

test this hypothesis, we prepared [U–^{2}H, ^{15}N]-chlorella ubiquitin in the presence of known inhibitors of transaminases (1 mM aminoxyacetate) and glutamate synthetase (0.1 mM L-methionine sulfoximine) during the translation reaction. The inhibitor concentrations used were those previously shown to inhibit transaminases (Morita et al. 2004). Expansions of 2D ^{1}H–^{13}C–HSQC spectrum show significant reduction in D → H back exchange at the α-positions of all residues (Fig. 3A) except for Gly (Fig. 3B). In addition, the inhibitors blocked back exchange at the β-positions of Ala (Fig. 3C). On the other hand, exchange at the β-positions of Asp and Asn, the γ-positions of Glu and Gln, and the ε-positions of Lys remained comparable to that observed for ubiquitin prepared in the absence of the inhibitors (Fig. 3D, E).

The percentage of back-exchange for deuterated ubiquitin prepared by wheat germ cell-free synthesis in the absence and in the presence of the inhibitors was estimated for those groups with isolated peaks by integration with SPARKY (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco). To determine the amount of the fully deuterated species present for each group (not visible in NMR spectra), the intensity of the peaks observed was compared with that of the corresponding peaks from an identical spectrum recorded on a fully protonated sample of [U–^{15}N] ubiqutin. To account for differences in sample and experimental conditions, the peak volumes were normalized on the basis of 2D ^{1}H,^{15}N–HSQC spectra acquired back-to-back for all samples on the same spectrometer using identical parameters. The results (Fig. 4A, B) clearly show that the most significant back-exchange occurs at the α-positions of Asp, Glu, Gln, Ala, and Gly, at the β-positions of Ala, Asp, and Asn, and, to a lesser extent, at the γ-positions of Glu and Gln and the ε-positions of Lys residues. Furthermore, the inhibitors effectively reduced the amount of back-exchange at the α-positions of all amino acids except for Gly and at the β-positions of only Ala. Back-exchange at all other positions was not significantly affected by the presence of the inhibitors.

Finally, for methylene groups that undergo back-exchange, we further investigated the percent population of the various protonated species observed (CH_2, CHD and CDH). In particular, we wanted to determine whether preferential exchange occurs at either of the prochiral positions. The results (Table 2) show that, although typically one position does exchange more than the other, the difference is significant only at the β-positions of Asn and

Table 2 Relative populations of protonated species in perdeuterated ubiquitin prepared in the absence and in the presence (second row, percent values shown in bold) of the inhibitors

			CH_2	CHD	CDH	CDH CHD[a]
Asp		D32/D39	38%	28%	35%	
			±2	±3	±1	
			41%	**25%**	**33%**	
			±1	**±1**	**±1**	
Asn		N25/N60	16%	24%	60%	
			±3	±2	±2	
			15%	**27%**	**58%**	
			±2	**±2**	**±2**	
Gln		Q62	18%	37%	45%	
			20%	**39%**	**41%**	
Glu		E34/E64	25%	35%	47%	
			±8	±7	±7	
			19%	**39%**	**47%**	
			±4	**±5**	**±5**	
Gly		G10/G76	40%	27%	33%	
			±5	±2	±3	
			33%	**35%**	**32%**	
			±7	**±4**	**±3**	
Lys		K6/K11	77%			23%
		K63	±1			±1
			75%			**25%**
			±6			**±6**

Data are shown only for groups exhibiting significant exchange with isolated peaks that could be easily integrated. The values were calculated such that all protonated species summed to 100%, i.e. the fully deuterated species is not taken into account. To see the fraction of protonated vs. deuterated species refer to Fig. 4. Columns headed by CHD/CHD_2 gives the percent protonation at the protonated sites in SAIL-ubiquitin

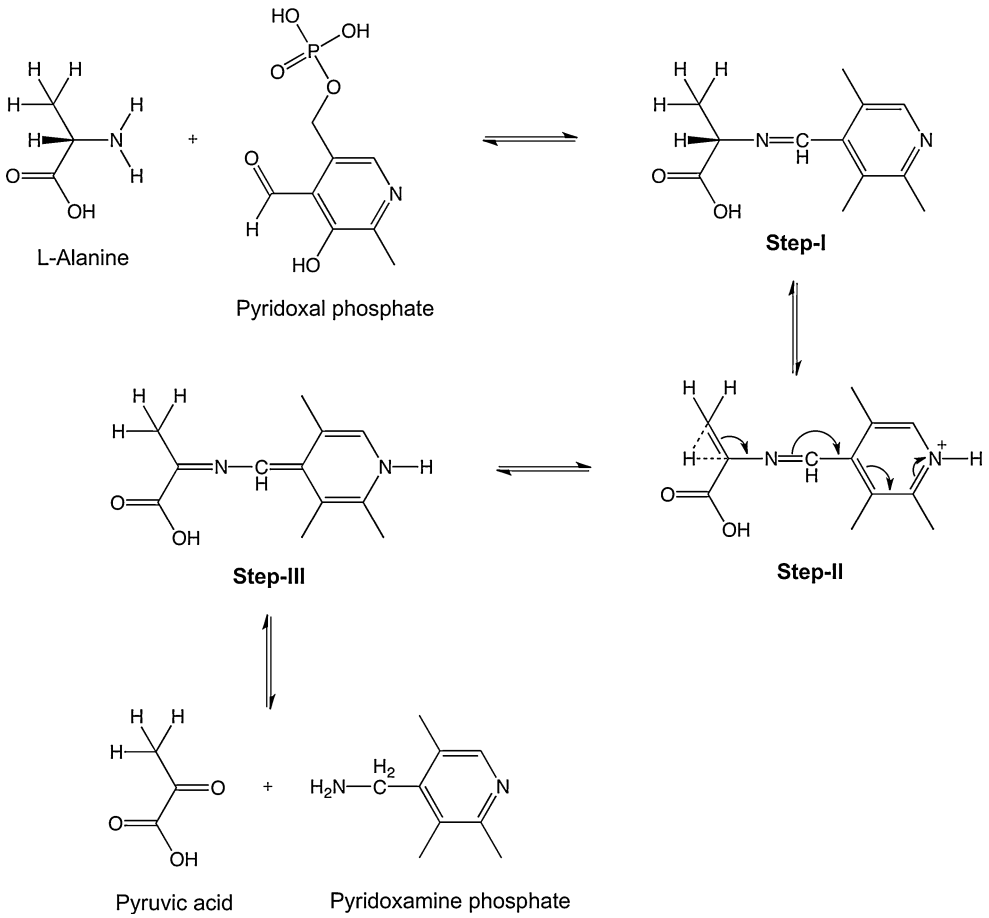
[a] The chemical shifts of the methylene protons are degenerate

to a lesser extent at the β-positions of Asp. Exchange effects appear to be similar in prokaryotic (*E. coli*) and eukaryotic (wheat germ) cell-free translation reactions, in that the prochiral positions that exhibited the highest back exchange in wheat germ synthesis were the same found to have exchanged in SAIL ubiquitin produced by *E. coli* cell-free synthesis: both resulted in the appearance of roughly equal peaks from single and double D → H exchange at β-positions (Table 1). We also observed that addition of the inhibitors did not affect the level of exchange at methylene sites (Table 2).

Alanine β-proton in transamination

A mechanism for the transamination reaction has been proposed that explains proton exchange at the α- and β-positions of Ala (Scheme 1) (Oshima and Tamiya 1961). During step I of the reaction, a Schiff base is formed between the amino acid and pyridoxal phosphate; subsequently a proton is lost from the β-carbon, and a hyperconjugated double-bond system is formed in the intermediate shown in step II. According to this scheme, a proton can then be incorporated from the aqueous solution at either the α- or β-position. This mechanism offers an explanation for the comparable levels of proton back-exchange that we observed at the α- and β-position of Ala residues (Fig. 4A, B). Furthermore, this mechanism also accounts for the decreased back-exchange we found when ubiquitin was prepared in the presence of the inhibitors, because blocking the transamination reaction would reduce proton back-exchange (Fig. 3C).

Scheme 1 Proposed transamination reaction mechanism leading to exchange at the H^{α} and H^{β} positions of alanine



Conclusions

We have used ubiquitin as a model for evaluating strategies for the preparation of a perdeuterated protein by incorporating ^{2}H-labeled amino acids with wheat germ cell-free translation. We found that in the absence of transaminase inhibitors, proton back exchange occurred at many sites, leading to signals from backbone α-protons and several side chain β-, γ-, and ε- protons. The addition of inhibitors to the wheat germ cell-free reaction blocked proton back exchange at the α-positions of all residues with the exception of Gly and at the methyl protons of Ala residues. However, partial back exchange continued to occur at several side chain sites. The patterns of exchange appear to be similar in the ubiquitin samples prepared by prokaryotic (*E. coli*) and eukaryotic (wheat germ) cell-free methods. We have not evaluated these inhibitors with *E. coli* cell-free protein production, but expect that they will be effective given the mechanistic conservation of the transamination reactions. We note that it may be possible to take advantage of these exchange reactions for the purpose of preparing perdeuterated proteins with selective protonation for NMR investigations of larger proteins, membrane proteins, protein–protein complexes, and protein-RNA complexes. While these studies were underway, it was reported that pretreatment with sodium borohydride of S30 *E. coli* extracts used for cell-free protein production can minimize proton exchange into the H^{α} positions of deuterated amino acids (Su et al. 2011). Sodium borohydride inhibits PLP-dependent enzymes; however, as shown here, selective inhibition of these enzymes did not prevent exchange at the H^{α} positions of Gly or various H^{β} positions.

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