A GENERAL PROCEDURE FOR THE PREPARATION OF α,β LABELED AMINO ACIDS

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SUMMARY

The α and β hydrogen exchange reactions catalyzed by pyridoxal have been applied to ten of the common amino acids to prepare α,β deuterated derivatives. The exchange and isolation procedure allows for numerous different samples to be processed in parallel. The deuteration at the α position range from 96 to 99% while the β deuteration levels range from 93 to 97% except for lysine (91%) which exchanges more slowly presumably due to interference of the ε-amino group. The standard procedure produces approximately a gram of deuterated product for all samples except tyrosine (0.15 g) with yields from 70 to 85% using only 15 milliliters of $^{2}H_2O$. The procedure has been scaled up directly to 100 millimoles for the case of alanine with virtually identical deuteration and product yield.

Key Words: Amino Acids, Deuteration, Pyridoxal Catalysis

INTRODUCTION

Specifically deuterated amino acids have proven to be of use in simplifying proton NMR spectra for dynamics measurements (1) as well as for homonuclear (2) and heteronuclear (3) spin coupling experiments. In its own right deuterium NMR is finding increasing use in biophysical studies (4). Work in our laboratory requires the synthesis of α,β deuterated derivatives of ^{13}C and ^{15}N labeled amino acids. A general deuterium exchange procedure which can be applied to ^{13}C or ^{15}N enriched amino acids offers obvious advantages over de novo synthesis from appropriately labeled precursors.

Abbott and Martell (5) observed that pyridoxal, in the presence of zinc (and other polyvalent) ions, catalyzes β hydrogen exchange as well as the previously characterized α hydrogen exchange (6). The synthetic

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potential of this reaction has been discussed previously but to date no general protocol has been published describing exchange conditions, isolation and product yield. The most successful effort directed at this problem was that of Tenenbaum et al. (7). Unfortunately their procedure as described is only applicable to freely soluble amino acids. By introduction of several modifications into their exchange procedure we have obtained efficient α,β exchange for ten different amino acids including tyrosine whose solubility is one thousand times less than that of alanine, the standard model amino acid. Only 15 milliliters of ^2H_2O is required for production of 5-10 millimoles of α,β deuterated amino acids in most cases. This procedure yields racemic mixtures as do most amino acid exchange procedures (note (8) for an exception).

RESULTS AND DISCUSSION

Tenenbaum et al. (7) exchanged the amino acids at a pD of 5.5 in the presence of pyridoxal and aluminum ions. The titration step can be circumvented by introducing sufficient pyridine to bring the pD to near the pK of that buffer (pK=5.21 in H_2O(9)). None of the amino acids have pK's near this value so that their buffering capacity is negligible. As a result, for the insoluble amino acids (tyrosine, phenylalanine and leucine) the pD of the solution is largely unaffected by the proportion of the amino acid in solution. Therefore the exchange can be effectively carried out on a suspension in which only a small proportion of the amino acid sample is in solution at any given time. Instead of refluxing under nitrogen as the earlier procedure suggests, the reactions were carried out in sealed glass ampoules which allowed higher temperatures (125°C) to be used and facilitated the handling of multiple samples in parallel.

Design of a general protocol for isolation of the deuterated product proved challenging mainly due to the zwitterionic nature of pyridoxal which causes its chromatographic and extraction behavior to be similar to that of the amino acids. Carrier displacement ion exchange chromatography using

volatile bases (10,11) provided a solution to this problem allowing efficient removal of pyridoxal except in the case of the basic amino acids lysine and arginine where an appreciable pyridoxal contaminant remains after chromatography. Since pyridoxal will not interfere with our later use no further effort was made to remove it. However, charcoal adsorbs pyridoxal so that such a treatment can be easily applied to remove residual contamination from the nonaromatic amino acid samples.

For each of the ten amino acids listed in Table I the level of deuteration was determined utilizing both ^1H NMR and mass spectral analysis (except for arginine for which only NMR analysis was conducted). The results can be briefly summarized as approximately 97% deuteration at the α position and 95% deuteration at the β position for each amino acid except for lysine for which the reaction proceeded more slowly presumably due to interference from the ε-amino group. The yields of isolated product (quantitated on a Durrum 500 amino acid analyzer) ranged from 70% to 85%. This level is not easily improved upon due to the loss of roughly 10% of the product which appears to remain in the extremely stable ternary pyridoxal-Al^{3+}-amino acid complex (12). For the case of alanine the procedure has been carried out on a 100 millimolar scale with a deuteration level and product yield as reported for the 10 millimolar scale.

The experimental procedure described for alanine is applicable to all of the amino acids listed in Table I except for glutamic acid and aspartic acid. The amount of pyridoxal hydrochloride, aluminum sulfate, sodium oxalate and pyridine in each case was scaled to the initial amino acid concentration as stated in Table I. The only variations required among the other seven amino acids are enumerated in that table.

Ten millimoles (0.40 g) of sodium hydroxide were added to the glutamic acid reaction to deprotonate the carboxyl group for the exchange step. The isolation procedure was also modified because a large proportion of the sample was converted to pyrrolidone carboxylic acid during the exchange reaction. The isolation and hydrolysis of the pyrrolidone carboxylic acid

TABLE I

REACTION CONDITIONS AND YIELDS FOR α,β DEUTRATION OF AMINO ACIDS

	Sample (mmoles)	Reaction Time (days)	Quench Volume (ml)	Carrier Base (mmoles)	HCl (mmoles)	Bed Volume (ml)	Displacer Concentration (M)	Extent of Deuteration (%) α	β	Yield (% - g)	
Alanine	10	2	200	pyr[a]-5	5	20	0.1	96	96	84	0.78
Arginine	10	2	200	col[b]-5	10	20	0.25	97	~95	85	1.50
Glutamic acid	10	2	200	pyr-5	10	25	0.1	97	95	80	1.20
Leucine	5	8	500	3mp[c]-2.5	3	10	0.05	98	97	69	0.46
Lysine	10	8	200	lut[d]-5	10	20	0.1	97	91	69	1.03
Methionine	10	2	200	pyr-5	5	20	0.1	97	97	79	1.20
Phenylalanine	5	8	500	3mp-2.5	3	10	0.025	98	95	78	0.65
Tyrosine	1	8	1000	3mp-1	1	4	0.01	99	97	80	0.15
Valine	10	4	200	pyr-5	5	20	0.1	98	93	78	0.93
Aspartic acid	10	2	200	pyr-5	17.5	30	0.05	99	97	77	1.05

[a] Pyrazole

[b] Collidine (2,4,6-trimethyl pyridine)

[c] 3-methyl pyrazole

[d] 2,6 lutidine (2,6-dimethyl pyridine)

is described in the Experimental section. Aspartic acid is quantitatively decarboxylated to alanine under the conditions described for alanine exchange so a two-step procedure was developed as described in the Experimental section. The exchange under basic conditions served to exchange the α hydrogen while the second acid step exchanged the β position which is activated by the carboxyl group (13).

The general procedure is inapplicable to cysteine (14), serine (15), threonine (16), histidine (17) and tryptophan (17) due to dominating side reactions as well as proline where the initial Schiff's base cannot be formed.

EXPERIMENTAL SECTION

Synthesis of DL-[α-^{2}H,β-^{2}H$_3$]alanine

Ten millimoles (0.891 g) of alanine, 1.0 millimoles (0.204 g) of pyridoxal hydrochloride and 0.25 millimoles (0.166 g) of $Al_2(SO_4)_3 \cdot 18H_2O$ were washed into a 25 ml glass bomb with 5 ml of ^2H_2O. The sample was vortexed vigorously and lyophilized. Five millimoles (0.42 ml) of pyridine and 10 ml of ^2H_2O were added to the sample. The vessel was frozen, evacuated to 50 μ Hg and sealed under vacuum. The ampoule was heated at 125°C for two days. An oil bath covered with aluminum foil was used to minimize photodecomposition of the pyridoxal.

The contents were washed into a 250 ml flask containing 200 ml of water, 2 millimoles of sodium oxalate, 5 millimoles each of pyrazole and of HCl. The resultant mixture should have a pH of 4 or less. After loading this solution onto a Dowex 50 column in hydrogen form (bed volume of 20 milliliters), the column was washed with water and the sample displaced with a 0.1 M NH_4OH solution. The initial ninhydrin positive fractions were pooled, rotary evaporated, redissolved in water and reevaporated to dryness to remove the pyrazole. The deuteration level was 96% at both α

and β positions and the yield of isolated product was 84% (0.78 g).

Isolation of DL-[α-^{2}H,β-^{2}H$_2$]glutamic acid

The reaction was quenched and the product was chromatographed as described for alanine. In addition the eluate from the sample loading and water wash was rotary evaporated to dryness, the residue was dissolved in 2 M HCl and refluxed for 2-3 hours (18). After the solution was evaporated to dryness, water was added and the sample was reevaporated twice to remove residual HCl. The residue was dissolved in 150 ml of water and loaded onto a 15 ml Dowex-50 column in hydrogen form. After washing the column with water, the sample was displaced with 0.1 M NH_4OH and the eluate was taken to dryness to remove the ammonia. The combined yield of the two glutamic acid samples was 80% (1.20 g).

Synthesis of DL-[α-^{2}H,β-^{2}H$_2$]aspartic acid

The initial steps of the alanine procedure were followed directly except that 20 millimoles (0.80 g) of sodium hydroxide were added to the initial sample. After lyophilization five millimoles (0.70 ml) of triethylamine were added instead of pyridine. The exchange was carried out at 50°C for 2 days. The quenching procedure and chromatography were conducted as for the alanine procedure with appropriate changes as indicated in Table I.

The resultant powder was dissolved in 8 ml of a 25% DCl solution and added to a glass ampoule which was then evacuated to 50 μ Hg and sealed. After heating at 125°C for eight days, the sample was diluted into water and rotary evaporated to dryness. Twice more water was added and the sample was taken to dryness to remove excess HCl. The deuteration level was 99% at the α position and 97% at the β position and the yield of DL-[α-^{2}H,β-^{2}H$_2$]aspartic acid was 77% (1.05 g).

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