

## Deuteration of Indole and N-Methylindole by Raney Nickel Catalysis

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### Summary

Indole and N-methylindole have been partially or fully deuterated by Raney nickel catalyzed  $^1\text{H}$ - $^2\text{H}$  exchange in a series of deuterated solvents. Perdeuterated indoles have been obtained in water and methanol while compounds that are preferentially deuterated at specific sites were obtained in chloroform, acetone, acetonitrile, ethanol, and isopropanol. The partially deuterated compounds are an important research tool for solid-state NMR studies on proteins.

**Keywords:** Deuterium, Raney nickel, deuteration, indole, N-methylindole, solid-state NMR

### Introduction

The amino acid tryptophan has attracted a lot of attention because of its role in the stabilization of transmembrane protein structure and its apparent involvement in specific interactions at ligand binding sites <sup>1-4</sup>. With the help of preferentially deuterated indoles, it is feasible to study protein conformation and dynamics by solid-state NMR.

Since its first application by Koch and Stuart <sup>5-7</sup>, the hydrogen-deuterium exchange reaction by Raney nickel catalysis has become the method of choice for specific deuteration of carbohydrates. The method has not been employed widely for deuteration of aromatic compounds because of non-selective deuteration and undesired reactions including deoxygenation of aldehydes and ketones, reduction of alkenes and alkynes, isomerization, polymerization, or oxygenation <sup>8-12</sup>. We investigated Raney nickel catalyzed hydrogen-deuterium exchange on the Trp analogs, indole and N-methylindole.

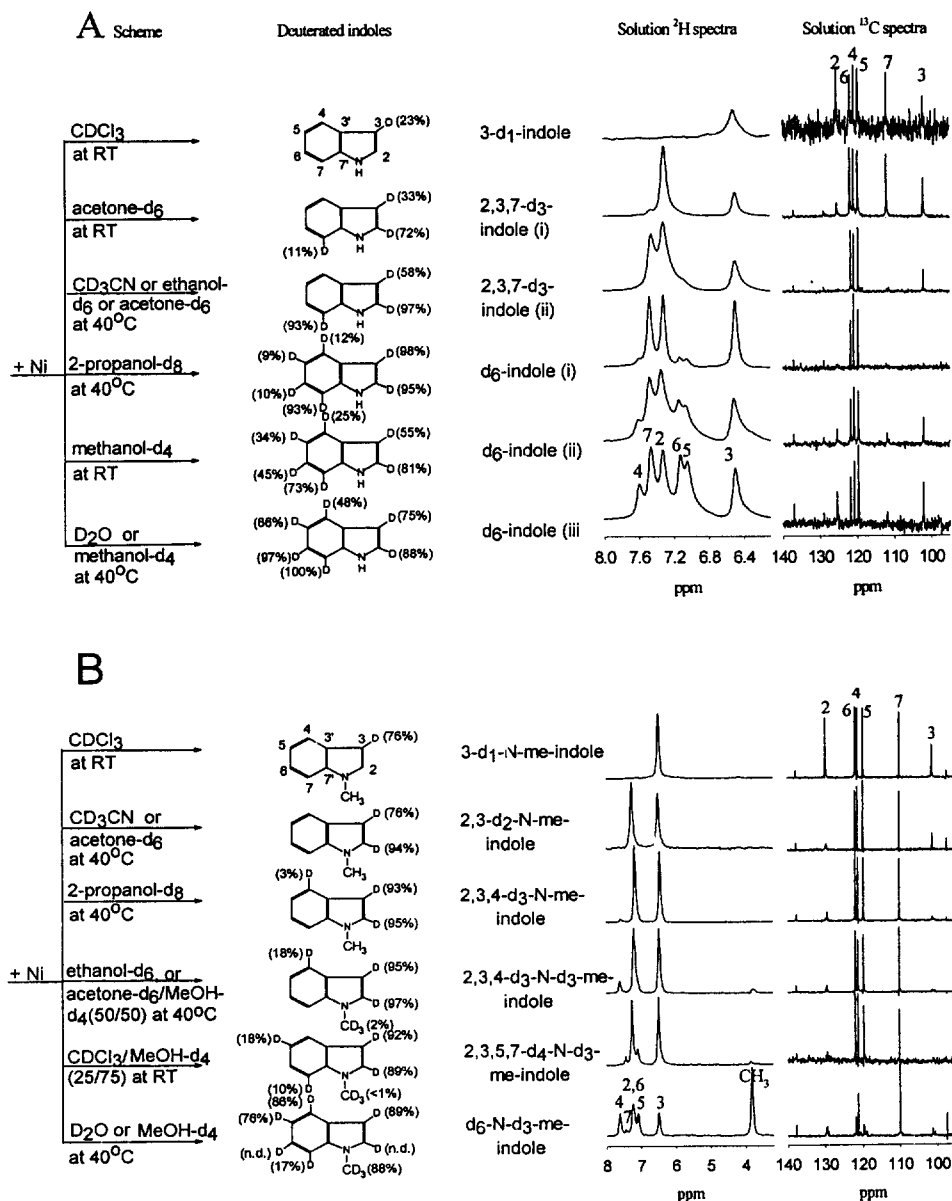


Figure. The specifically deuterated indoles (A) and N-methylindoles (B) have been prepared by  $^1\text{H}$ - $^2\text{H}$  exchange reaction of indole with Raney nickel (Ni) in deuterated solvent at room temperature (RT) or 40°C (synthetic scheme shown on the first column). The signal assignments for indole and N-methylindole proton and carbon resonances were made by 2D-COSY and 2D-HETCOR. The degrees of deuteration (provided in the second column) was determined by solution  $^1\text{H}$ - $^2\text{H}$  (46.1 MHz, in protonated acetone with D<sub>2</sub>O as reference at 4.8 ppm, expanded spectra shown on the fourth column) and  $^{13}\text{C}$  NMR (75.5 MHz, in acetone-d<sub>6</sub> at 29.8 ppm, expanded spectra shown on the last column) experiments. Note: n.d. - not-determined.

## Results and Discussion

*Non-selective hydrogen-deuterium exchange in deuterium oxide.* Perdeuterated Trp analogs were obtained by treatment with deuterium oxide at 40°C. Under these mild conditions, no selective deuteration or reduction takes place. Reduction of aromatic compounds has been observed under harsher conditions such as 24 hour reflux in deuterium oxide or heavy water with 10 % w/v Na<sub>2</sub>CO<sub>3</sub><sup>8</sup>.

*Selective deuterium exchange in alcohols.* The degree of deuteration was somewhat dependent on the size of the alcohols (compare methanol, ethanol and isopropanol on Schemes of Figures A & B). Complete deuteration is achieved by reaction in methanol while ethanol and isopropanol resulted in partial deuteration. The role of alcohols as hydrogen (in this case, deuterium) donors has been discussed but the need for such a donor has been questionable since partial deuteration was obtained in aprotic solvents as well<sup>13,14</sup>.

*Highly selective deuterium exchange in an aprotic solvent.* When the exchange reaction took place in acetone, acetonitrile or chloroform, the pyrrole ring protons, except the carbon 7 proton of indole, were more susceptible to exchange than the benzene ring protons. Acetone and, perhaps, acetonitrile may serve as a hydrogen (or deuterium) acceptor in deuterium exchange with Raney nickel<sup>13</sup>.

Previous studies in aromatic compounds such as aromatic acids, alcohols or ketones showed that Raney nickel is able to catalyze deuterium exchange of aromatic ring protons<sup>8,10-12</sup>. This communication shows that deuterium exchange also occurs on Trp-analogs such as indole (Figure A), N-methylindole (Figure B) and 3-methylindole (data not shown). In order for this exchange reaction to take place, the Trp-analogs must first adhere to the catalytic surface. Methylation of the nitrogen atom does not inhibit Raney nickel-catalyzed exchange demonstrating that the presence of an imino group as a chemisorption site is not essential<sup>5,9,15,16</sup>. Instead, we suggest that the strong electric dipoles and quadrupoles of aromatic ring systems are responsible for the surface attachment. It has been demonstrated that strong attractive forces exist between cations and aromatic rings<sup>17,18</sup>. It is possible that the Trp-analogs are attracted to the catalyst by the charges on its surface.

Deuteration specificity may be attributed to the relatively high nucleophilicity of carbon 3 and, perhaps, carbon 2<sup>19-21</sup>. In addition, solvent physicochemical properties such as polarity may influence Trp-analog attachment to the catalytic surface. We propose that indole ring orientation, and consequently the reactivity of indole ring protons, are sensitive to the complex electrical fields at the catalytic surface. In turn, the changes in these fields by the presence of solvents could result in deuteration specificity.

Tryptophan that has been deuterated enables determination of assigned  $^2\text{H}$  NMR order parameters to study conformation and flexibility at distinct protein sites. The preferentially deuterated indoles can be readily incorporated into any polypeptide or protein of interest<sup>22,23</sup>. We used deuterated Trp analogs obtained from the reactions of Raney nickel to determine differences in orientation and mobility between the Trp analogs in membranes (Yau *et al.*, in preparation).

### General Method

Deuterated Raney nickel catalyst was prepared as described by Lockley *et al.*<sup>10</sup>. Indole or N-methylindole (0.4 mmol) was dissolved in deuterated solvent (1.5 ml) and then mixed with dry deuterated Raney nickel (approximate 2.0 g). The exchange reaction was conducted over a week at room temperature, or 40°C, as specified in the schemes of Figures A and B. The residual nickel was filtered off at the end of the reaction and washed three times with petroleum ether (10.0 ml). After removal of the organic solvent by rotary evaporation, water (20.0 ml) was added to the residue and the raw product was extracted by petroleum ether (10.0 ml). The deuterated indole was recrystallized in a water-methanol mixture whereas the deuterated N-methylindole was purified by distillation (bp<sub>20</sub> 120°C) or column chromatography using methanol/petroleum ether (1/6, R<sub>f</sub> = 0.5). This procedure gave pure, preferentially deuterated products in a quantitative yield.

### References

1. Landolt-Marticorena, C., Williams, K.A., Deber, C.M. & Reithmeier, R.A.F. *J.Mol.Biol.* **229**, 602-608 (1993).
2. Hu, W. & Cross, T.A. *Biochemistry* **34**, 14147-14155 (1995).
3. Maruyama, T. & Takeuchi, H. *Biochemistry* **36**, 10993-11001 (1997).
4. Tsuboi, M., Overman, S.A. & Thomas, G.J.J. *Biochemistry* **35**, 10403-10410 (1996).
5. Koch, H.J. & Stuart, R.S. *Carbohydr.Res.* **59**, C1-C6 (1977).
6. Koch, H.J. & Stuart, R.S. *Carbohydr.Res.* **64**, 127-134 (1978).
7. Koch, H.J. & Stuart, R.S. *Carbohydr.Res.* **67**, 341-348 (1978).
8. Pojer, P.M. *Tetrahedron Lett.* **25**, 2507-2508 (1984).
9. Harness, J. & Hughes, N.A. *J.Chem.Soc.Perkin I.* 38-41 (1972).

10. Lockley, W.J.S. & Kershaw, G. *Synthesis and Applications of Isotopically labelled Compounds* 1991 in Buncel, E. & Kabalka, G.W. (Eds.) 604-607, Elsevier Science Publishers, B.V., 1992.
11. Tashiro, M., Nakayama, K. & Fukata, G. *Chem.Lett* 359-362 (1981).
12. Tashiro, M. & Nakayama, K. *J.Chem.Soc.Perkin Trans.I.* 2315-2318 (1983).
13. Chakravarti, R.N. *J.Indian Chem.Soc.* **LII**, 1-3 (1975).
14. Karabinos, J.V. *Methods Carbohydr.Chem.* 77-79 (1963).
15. Balza, F. & Perlin, A.S. *Carbohydr.Res.* **107**, 270-278 (1982).
16. Angyal, S. & Stevens, J.D. *Carbohydr.Res.* **157**, 83-94 (1986).
17. Mecozzi, S., West, A.P. Jr., & Dougherty, D.A. *Proc.Natl.Acad.Sci.U.S.A.* **93**, 10566-10571 (1996).
18. Dougherty, D.A. *Science* **271**, 163-168 (1996).
19. Pachter, R., Woudenberg, M.E.D. & Wessels, P.L. *S.Afr.Tydskr.Chem.* **40**, 172-178 (1987).
20. Terrier, F., Pouet, M.-J., Halle, J.-C., Hunt, S., Jones, J.R. & Buncel, E. *J.Chem.Soc.Perkin Trans.II.* 1665-1672 (1993).
21. Challis, B.C. & Millar, E.M. *J.Chem.Soc.Perkin II.* 1618-1624 (1972).
22. Murakami, Y., Hisaeda, Y., Miyajima, T., Sakata, H. & Kikuchi, J.-I. *Chem.Lett* 645-648 (1993).
23. Weiner, W., Winkler, J., Zimmerman, S.C., Czarnik, A.W. & Breslow, R. *J.Am.Chem.Soc.* **107**, 4093-4094 (1985).