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Solvent-Induced Control Of Ultrasonic Deuterium Labelling

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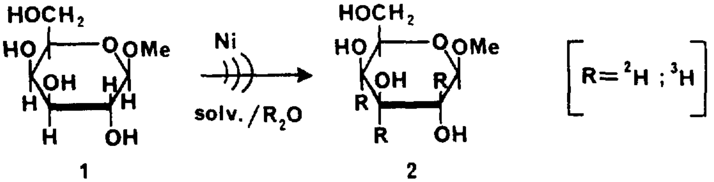
Abstract: Raney Nickel catalyzed hydrogen-deuterium exchange in a model monosaccharide was conducted in eight different solvent pairs under ultrasonic irradiation. Immiscible solvent pairs gave percentages of stereospecific deuterium incorporation comparable to those reported previously; pronounced regioselectivity was demonstrated in two of the pairs examined. Copyright © 1996 Elsevier Science Ltd

Exchange reactions between isotopic hydrogen donors and organic substrates remains an important goal in biosynthetic and organic synthetic chemistry. In particular, deuterium and tritium labelling of simple to complex carbohydrates, and carbohydrate moieties of glycoproteins and glycolipids, continues to engender major efforts in homogeneous and heterogeneous catalysis. Isotopic labels serve as convenient probes into the molecular organization and structural dynamics of micelles and cellular membranes.[1] Further, deuterium and tritium labels are commonly used to follow and elucidate cellular metabolic and biosynthetic pathways and their associated activities.[2]

Recent efforts have resulted in the further development of a new facile technique,[3] which affords stereospecific H → D (or H → T) exchange, under very mild conditions, which is facilitated by ultrasonic irradiation (<u>S</u>tereospecific <u>U</u>ltrasonically <u>P</u>romoted <u>E</u>xchange <u>R</u>eaction, or "S.U.P.E.R."). Ultrasonic irradiation affords a significant amelioration in the ability to promote stereospecific hydrogen-deuterium exchange, without the attendant degradation or racemization of products as observed by others when conducted at higher temperatures and long incubation periods.[4] Indeed, the S.U.P.E.R. procedure has been successfully utilized for rapid labelling of a model carbohydrate (1-O-methyl-β-D-galactopyranoside; c.f. 1 → 2),[3]



1 2

a thermally sensitive glycosphingolipid,[3] free nucleosides of DNA and RNA,[5] synthetic glycolipid analogues,[6] and the methyl glycosides of N-acetylneuraminic acid (a sialic acid prevalent in animal tissues).[7] Further investigations have extensively examined the surface and bulk morphological properties of several Raney-type catalysts.[8,9] These studies revealed that, as opposed to any direct cavitational phenomena (such as thermal or pressure effects or increased bulk transport),[8] ultrasonic irradiation promotes bulk crack-propagation,[9] exposes and defines distinct catalytic sites,[8] removes passivating impurities,[8] and induces both elemental and ionic redistribution within the catalyst bulk itself.[8,9]

Earlier work has shown that a solvent pair consisting of THF and D_2O, in a 5:1 ratio, to be very effective for catalytic H → D exchange.[3] As seen for other organic substrates, the rate of H → D exchange proceeds at rates comparable to D_2O alone.[10] Although this miscible solvent pair will solvate many natural products (or adducts of same), one may envision difficulties when dealing with larger, more complex biomolecules such as oligosaccharides, glycoproteins, glycolipids, etc. Further, cost, safety, and disposal considerations will dictate that a minimal amount of tritiated water be used for selective H → T exchange reactions when using this technique. As an aside, it may be possible that modifications of the solvent pair(s) employed may aid in the regioselective control of the exchanging sites. Such control, when coupled with the demonstrated stereospecificity of this exchange technique, would indeed be desirous and of obvious synthetic utility.

Experiments were conducted as outlined previously,[3] utilizing singularly the solvents listed in Table 1 along with D_2O in a 5:1 solvent/D_2O ratio as before. All solvents were reagent grade or better, and utilized as received. In a typical experiment, a 3-necked 25 mL flask equipped with a central ground-glass joint (~14 cm long) was charged with 20 mg of substrate, 0.5 mL (settled volume) of deuterated Raney nickel,[3] 10 mL of organic solvent, and 2 mL of D_2O. The flask was immersed in a thermostatted water bath (ca. 40°C), and the flask contents swept gently with argon. Sonication was conducted for 2 hours using either a Bransonic Model W-200 P or a Bransonic Model 250 sonicator, equipped with a titanium tip, exactly as before;[3] the deuterated substrate was isolated as discussed previously.[3]

The levels of deuterium incorporation ($\underset{\sim}{1}$ → $\underset{\sim}{2}$) were followed by monitoring the disappearance of signals (and relevant changes in multiplicities) in the ^1H NMR spectra.[3] The eight solvent mixtures employed were not exhaustively examined with regards to specific H → D exchange reproducibility, since prior work had shown that continuous ultrasonication of THF / D_2O mixtures utilizing different catalyst batches to be very highly reproducible, as adjudged by both initial and relative kinetic rates.[8]

Table 1 lists the relative percentages of deuterium incorporated into each of the exchangeable sites of the model carbohydrate, 1-O-methyl-β-D-galactopyranoside, within two hours of continuous ultrasonication over Raney nickel:

TABLE 1

Percentage of ^{2}H Exchange in 1-O-Methyl-β-D-galactopyranoside After 2 Hrs Continuous Ultrasonication

	THF	DMTHF	THP	MDOX	PDOX	DME	DNBE	HPT
C_2	27	28	20	25	<1	15	22	20
C_3	73	84	59	60	62	<1	74	90
C_4	89	93	59	76	75	29	63	85

Solvent-pair with D_2O:
THF = Tetrahydrofuran
DMTHF = 2,5-Dimethyltetrahydrofuran (emulsion)
THP = Tetrahydropyran (emulsion)
MDOX = 1,3-Dioxane
PDOX = 1,4-Dioxane
DME = 1,2-Dimethoxyethane
DNBE = Di-n-butyl ether (emulsion)
HPT = n-Heptane (emulsion)

Noteworthy is the observation that *the solvent pairs need not be miscible for successful H → D exchange!* Indeed, a solid suspension of catalyst in a mixed-pair emulsion afforded levels of ^{2}H incorporation similar to the totally miscible THF/D_2O solvent pair examined in earlier work.[5] Most surprisingly, however, was the finding that in two cases different exchange sites could be effectively "switched-off" by use of an appropriate solvent pair. In none of the experiments was ^{2}H incorporated at C_6, nor were any degraded byproducts or racemized materials isolated as adjudged by both ^{1}H NMR spectroscopy and thin layer chromatography (TLC).

The rationale for the observed mediation of the exchange regioselectivity seen with two of the solvent pairs examined remains somewhat elusive at this time. There is no apparent correlation of the selectivity with the usual solvent parameters such as densities, dielectric constants, vapor pressures, viscosities, polarity indexes, etc.[11] One plausible explanation may be that the hydrocarbon co-solvents (c.f., PDOX and DME, Table 1) competitively interact or block active exchange sites on the catalyst surface itself. Nonetheless, this study has further illustrated the potential utility of the S.U.P.E.R. technique for facile isotopic exchange in more complex, sparingly-soluble (or aqueously insoluble) biomolecules, as well as revealing the unique possibility of solvent-mediation of regioselectivity.

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