INVESTIGATION ON SOLVENT HYDROGEN TRANSFER DURING THE CATALYTIC DEUTERATION
OF N-ACETYL-3,5-DI-IODO-L-TYROSINEAMIDE

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Summary

   During the catalytic dehalodeuteration of N-acetyl-3,5-di-iodo-L-tyrosine
amide solvent hydrogen was incorporated instead of deuterium up to an order
of 70%. The major part of the solvent hydrogen was shown to be introduced
into the reaction product independently of the dilution of the reacting gas.
This direct transfer depends on type of solvent and catalyst and on the
catalyst-to-substrate ratio in the same manner as found from deuterations
of 4-halogenated phenylalanine derivatives and dehydroproline. The distri-
bution of deuterium in the reaction products was determined by mass spec-
trometry.

Key words: Catalytic deuteration, solvent hydrogen transfer, mass spectro-
           metry

Introduction

   Catalytic tritiations are accompanied more or less by incorporation of
hydrogen. Even the use of conditions proved for minimum dilution of tritium
gas by hydrogen from solvent /1/ does not warrant reliable labelling results.
A catalyst-mediated transfer of solvent hydrogen independently of the dilution
of the reacting gas, proposed by Marton and Kovacs /2/, has been considered so
far to be of small importance. In catalytic deuteration experiments with

derivatives of 4-halogenated phenylalanine /3/ and dehydroproline /4/ we
found such direct transfer to be as important for the labelling result as
the dilution of the reacting gas by solvent hydrogen.

Here we present results from a more detailed investigation of a catalytic
dehalodeuteration using N-acetyl-3,5-di-iodo-L-tyrosineamide (Ac-Dit-NH$_2$) to
confirm the previous findings. For the purpose of comparison we applied the
same catalysts and analogous reaction conditions as in the foregoing studies
/3,4/.

Experimental

N-Acetyl-3,5-di-iodo-L-tyrosineamide (Ac-Dit-NH$_2$) was obtained by acety-
lation of 3,4-di-iodo-L-tyrosine (Fluka, Switzerland) followed by SOCl$_2$/CH$_3$OH-
esterification and amidation in NH$_3$/CH$_3$OH. C$_{11}$H$_{12}$I$_2$N$_2$O$_3$, m.p. 206-208 °C
(0.1 N NH$_3$), $| \alpha |_D^{20}$ = 13.8 (c=1; CH$_3$OH).
N-Acetyl-L-tyrosineamide, (Ac-Tyr-NH$_2$) C$_{11}$H$_{14}$N$_2$O$_3$, m.p. 224-226 °C
(0.1 N NH$_3$) was prepared analogous.

The catalysts, palladium 10 % on Al$_2$O$_3$ and rhodium 5 % on Al$_2$O$_3$ were pur-
chased from Fluka (Switzerland). Dimethylacetamide (DMA) (Merck-Schuchard, FRG)
and deuterated Dimethylacetamide-D$_9$ (Cambridge Isotope Laboratories, Woburn,
Mass. USA) were distilled and treated successively with molecular sieves 10 X
and 3 A.

The deuterium was produced by electrolyzing D$_2$O ($>$ 98%, containing 5 %
H$_2$SO$_4$ on Pt-electrodes.

The hydrogen content in D$_2$/H$_2$-mixtures was estimated from the D$_2$O content
in the electrolyzing mixture using an isotopic effect of 1.5 for the electro-
lysis /4/.

The deuterations or hydrogenations were performed in a rectangular 20 ml
Warburg-vessel equipped with an additional side-bulb at ambient temperature,
normal pressure and a constant flow of deuterium (100 ml/h). The substrates,
(see Tables) dissolved in 1.4 ml of the solvent, and 50 mg of the catalyst
were placed separately into the chambers and combined after 1 h presaturation.
The reaction was complete within 0.5 h except in DMA and DMA/D$_2$O 1.38/0.02 at
low Pd/substrate ratios. After shaking one hour the catalyst was removed by
centrifugation and washed with CH$_3$OH. After evaporation the residue was
dissolved in CH$_3$OH/H$_2$O, reevaporated twice, and directly used for mass
spectrometric measurements.

After reaction in the presence of tritiated water (50 - 1500 MBq),
200 mg of N-acetyl-L-tyrosineamide were dissolved in an aliquot of the
catalyst free reaction solution, the fivefold amount of water was added

and the mixture was allowed to crystallize. The recrystallisation was repeated from DMA/H_2O 1/5 until the specific radioactivity of the substance (estimated by liquid scintillation counting and weighting) remained constant (at least five times).

The absorption of the reaction product on the catalyst was lower than 10%, as estimated photometrically after reaction with diazotized sulfanilic acid in analogy to /5/.

An exchange of carbon-bound tritium during the recrystallisation procedures could be excluded by refluxing an analogously deuterated sample for 1 h in DMA/H_2O 1/5. No loss of deuterium could be detected.

To complete the estimation of the solvent radioactivity, after the reaction the catalyst metal was dissolved by heating with conc. nitric acid and the solution was counted additional.

The mass spectra were recorded on a MS 902 S-spectrometer (AEI, Manchester, England) at 70 eV. The degree of deuteration was determined by means of the intensities of the peaks m/z 222 - 226, corrected for the natural isotope abundance, estimated in the mass spectrum of Ac-Tyr-NH_2. The spectra of both the reference and the deuteration products were recorded under the same conditions, and the intensities were determined as an average of five recordings of the samesample.

Results and discussion

After dehalodeuteration of Ac-Dit-NH_2 in the presence of tritiated water we observed incorporations of tritium into the reaction products up to an order of about 90% of the specific radioactivity of the used labelled water (table 1). However we could not detect any loss of radioactivity in the solvent after the reaction (taking into account a measuring error of about $\pm$ 5%). This fact does present strong evidence for a direct incorporation of sol- vent hydrogen into the reaction product according to /2/ and confirms earlier findings /3,4/. If a dilution of the reacting gas by solvent hydrogen would be the major mode for the introduction of the tritium found in the reaction products (table 1), then a loss of the solvent radioactivity of at least 10 - 20 percent should be observed under the reaction conditions used here (flowing gas, 1 mmol solvent water, 20 ml gas volume; see Experimental). Further evidence for a transfer of the solvent hydrogen directly to the substrate is provided by the fact that the replacement of the deuterium gas

by hydrogen did not affect the specific radioactivity of the reaction product, whereas the use of H_2O instead of D_2O in the solvent diminished the incorporation of tritium into the reaction product to about one-half (table 1).

Table 1: Specific radioactivity of Ac-Tyr-NH_2 in percent of the specific
radioactivity of tritiated water contained in the solvent obtained
after 1h catalytic deuteration of Ac-Dit-NH_2 in the presence of
Pd/Al_2O_3 10%.

Solvent	mol Pd/ mol sub- strate	Specific radioactivity of the reaction product in percent of the specific radioactivity of the solvent water
1.38 ml DMA + 0.02 ml		
$^3H_2O/D_2O$	5	5.3
$^3H_2O/D_2O$	25	9.0
$^3H_2O/D_2O$	50	11.8
$^3H_2O/D_2O$	50[a]	11.0
1.38 ml DMA + 0.02 ml		
$^3H_2O/H_2O$	50	4.6
0.7 ml DMA + 0.7 ml		
$^3H_2O/D_2O$	50	91.0

a) use of hydrogen gas

A passage of the tritium into the gas volume before the reaction with the substrate should result in different specific radioactivities of the reaction products if hydrogen is used instead of deuterium gas, due to different isotopic effects.

Coupling of the reaction products with a treefold excess of diazotized sulfanilic acid liberated more than 60 % of the tritium, showing its predominant incorporation in position 3 and 5 of the ring.

In order to confirm earlier results on hydrogen transfer not only from water but even from an aprotic solvent /3,4/ Ac-Dit-NH_2 was dehalohydrogenated catalytically in deuterated DMA (DMA-D_9). Table 2 shows that hydrogen can be

transferred in a considerable extent also from an aprotic solvent to the

substrate. The deuteration degrees obtained in the presence of rhodium

(table 2) imply preference of a direct transfer of the solvent hydrogen also

in this case. For transfer of such an amount of deuterium

Table 2: Mass spectrometric results after catalytic dehalogenation of
Ac-Dit-NH_2 using different D/H-mixtures and deuterated DMA in
the presence of Pd/Al_2O_3 10 % or Rh/Al_2O_3 5 % (mol Pd/mol
substrate: 5/1; mol Rh/mol substrate 2.5/1).

Solvent	cata- lyst	% H_2 in the D_2- gas .	$\dfrac{I_{corr.m/z}\ x\ .\ 100}{\Sigma\ I_{corr.m/z}\quad 222 - 225}$ [a]				D/mol
			x=222 $(^2H_0)$	x=223 $(^2H_1)$	x=224 $(^2H_2)$	x=225 $(^2H_3)$	
DMA/D_2O 1.38/0.02	Pd	24	47.4	41.3	10.4	0.8	0.65
DMA/D_2O 1.38/0.02 [b]	Pd	24	68.5[b]	31.5[b]	–	–	0.32
DMA/D_2O 1.38/0.02	Pd	50	64.8	31.0	4.2	–	0.39
DMA-D_9/H_2O 0.7/0.7	Pd	100	93.2	6.8	–	–	0.07
DMA-D_9/H_2O 1.38/0.02	Rh	100	70.8	24.7	3.7	0.8	0.35
DMA-D_9/D_2O 1.38/0.02	Rh	100	49.1	38.8	10.9	1.2	0.64

a) $I_{corr.m/z}x$ = Intensity of the ms peak appearing at the mentioned mass number,
diminished by the intensity of the natural isotopic peak related
to x-1 and x-2, which was estimated by means of the mass spectrum
of nondeuterated Ac-Tyr-NH_2

 b) for deuterated N-acetyl-3-iodo-tyrosineamide, contained in the reaction pro-
duct, determined as described for the deuterated Ac-Tyr-NH_2 by means of the
peaks at m/z 348-350 ($^2H_0$-$^2H_2$) in the mass spectrum

across the gas phase a deuterium content in the reacting gas of at least 50%

would be needed according to the experiments with different HD-mixtures

(table 2). However, such a high rate of gas-solvent exchange should result in

the reversed deuteration using nondeuterated DMA in clearly lower deuteration

degrees than were found (table 3) due to an isotopic effect k_H/k_D of about 7

(see below).

Table 3: Mass spectrometric results after catalytic deuteration of Ac-Dit-NH$_2$
in the presence of Pd/Al$_2$O$_3$ 10% or Rh/Al$_2$O$_3$ 5%

Solvent	mol Pd or mol Rh* per mol sub- strate	$\frac{I_{corr.m/z}x \cdot 100^{a)}}{\Sigma \ I_{corr.m/z} \ 222-226}$					D/mol
		x=222 (^{2}H$_0$)	x=223 (^{2}H$_1$)	x=224 (^{2}H$_2$)	x=225 (^{2}H$_3$)	x=226 (^{2}H$_4$)	
DMA	5	3.3	16.0	73.7	6.3	0.8	1.86
DMA/D$_2$O 1.38/0.02	5	3.7	13.0	78.5	3.8	1.0	1.86
DMA/D$_2$O 1.38/0.02	25	2.3	13.8	67.6	14.4	1.9	2.00
DMA/D$_2$O 1.38/0.02	50	2.8	16.6	60.1	17.4	3.2	2.02
DMA/D$_2$O 0.7/0.7	5	2.8	10.6	70.6	14.3	1.7	2.02
DMA/H$_2$O 1.38/0.02	5	4.2	20.9	66.6	6.8	1.6	1.81
DMA/H$_2$O 0.7/0.7	5	7.6	33.7	51.7	6.0	1.0	1.59
DMA/0.2M HCl 0.7/0.7	5	13.9	41.8	39.4	3.2	1.8	1.37
DMA/0.2M Et$_3$N 0.7/0.7	5	9.1	29.6	59.3	1.7	0.3	1.55
DMA/H$_2$O 0.7/0.7 [b)]	5	58.9	32.6	7.6	0.8	–	0.50
DMA/D$_2$O 0.7/0.7[b)]	5	32.3	46.1	18.3	2.8	0.5	0.93
DMA	2.5*	9.9	39.5	45.0	4.1	1.6	1.48
DMA/D$_2$O 1.38/0.02	2.5*	6.4	32.6	54.9	4.8	1.4	1.62
DMA/H$_2$O 1.38/0.02	2.5*	53.1	34.8	10.3	1.9	–	0.61
DMA/H$_2$O 0.7/0.7	2.5*	57.5	33.3	4.7	4.5	–	0.56

a) see footnote a) of table 2

b) reaction of the noniodinated Ac-Tyr-NH$_2$

The incorporation rates of solvent hydrogen into the tyrosine derivative
(taken from the ^{2}H$_0$- and ^{2}H$_1$-species in the mass spectra) are influenced by
the type of solvent and catalyst and by the catalyst-to-substrate ratio in a
similiar manner as has been found in the deuteration of halogenated phenyl-
alanines /3/ and dehydroproline /4/ (tables 1-3). This underlines a more
general importance of the observed effects even for catalytic tritiations
using both halogenated precursors and unsaturated compounds. The same can be
stated concerning the nonspecific introduction of deuterium additionally to

the replacement of the iodine (taken from the $^2H_3$- and $^2H_4$-species in the mass spectra).

In difference with the earlier studies for Ac-Dit-NH$_2$ a 5-10fold catalyst-to-substrate ratio was required to reach deuteration rates comparable with those for both the halogenated phenylalanines and dehydroproline /3,4/.

The isotopic effect k_H/k_D of 5-7, derived from the experiments using different DH-mixtures (table 2), differs noticeably from the value of about 2 found for the catalytic deuteration of the 4-halogenated phenylalanines /3/. One reason for this discrepancy might be the presence of the hydroxyl group of tyrosine. The second halogen in di-iodotyrosine cannot be responsible for the difference, because the same isotopic effect can be derived for the partial dehalogenation leading to the monoiodinated derivative (table 2).

After presaturation of the catalyst in the solvent only 60% of the deuteration degree could be achieved as obtained under the same conditions but presaturating the dry catalyst. Only after presaturating of the dry catalyst (table 3) the pH affected noticeably the deuteration result.

During the catalytic deuteration of the noniodinated tyrosine derivative (table 3) a remarkable exchange of carbon-bound hydrogen takes place. Such exchange should also affect the labelling result of the catalytic deuteration of Ac-Dit-NH$_2$. Surprisingly we could not find any difference in the labelling results after catalytic deuteration of Ac-Dit-NH$_2$ in DMA/H$_2$O 1/1 for 30, 60 (table 3) or 120 minutes. This result provokes the assumption that the exchange proceeding after the dehalogenation takes place preferably in the 3 and 5 positions of the aromatic ring and not in the benzylic position of the tyrosine molecule according to Evans et al. /6/. The same conclusion should be valid to explain the only slight enhancement of the deuteration degree after reaction in the presence of D$_2$O (table 3).

Conclusions

An important part of the undesired incorporation of solvent hydrogen into the reaction product during catalytic deuterations and, therefore, very likely also during catalytic tritiations proceeds in a direct way and may not be influenced by methods affecting a dilution of the reacting gas. This

direct incorporation of solvent hydrogen can be minimized simply by minimizing the catalyst-to-substrate ratio, avoiding of protic solvents and evaluation of the catalyst not only by its hydrogenation activity but also by its ability to introduce solvent hydrogen into the substrate. Another drawback encountered generally with catalytic tritiations, the position-unspecific labelling, can be minimized accordingly.

On the basis of our deuteration results it seems unlikely to avoid an incorporation of solvent hydrogen completely and to achieve position-specific labelling degrees higher than about 80% in catalytic tritiations. Higher specific activities should be attainable by a nonspecific labelling in additional positions.

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