

INVESTIGATION OF THE CATALYTIC DEUTERATION OF N-ACETYL-DL-3,4-DEHYDROPROLINE AMIDE

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Summary

As a model for the tritium labelling of peptides the catalytic deuteration of N-acetyl-DL-3,4-dehydroproline amide was investigated by means of mass spectrometric measurements. The unwanted incorporation of hydrogen instead of deuterium, found especially in the presence of water, was compensated partially by an incorporation of deuterium proceeding additionally to the saturation of the double bond. Both effects were most intensive in the presence of water and rose with ascending catalyst amounts and descending pH.

Key words: Catalytic deuteration, solvent hydrogen transfer, mass spectrometry

Introduction

In spite of a great number of catalytic peptide tritiations reported up to now, no general guidelines for optimal labelling conditions can be extracted from these reports. Very different results, ranging from beyond 10 percent of the theoretical achievable specific radioactivity up to the theoretical ones in the labelling product, were described, and no general correlation between the results and the type of solvent or catalyst or the structure of the precursor can be derived. Systematic studies, dealing with factors which

can be of influence for the incorporation of hydrogen competing with the wanted incorporation of tritium, are found rarely, probably because of the efforts and cost connected with tritiations and due to the complexity of heterogeneous catalysis in general.

To contribute to a better understanding in this field, we have studied in a previous work the catalytic deuteration of halogenated phenylalanine derivatives as a model for one of the two reaction types used most widely in peptide tritiations /1/. According to the results of these model investigations and in contrast to the situations mentioned before it should be possible to give general guidelines for minimizing the unwanted incorporation of hydrogen in dehalo-tritiations.

To find out if analogous conclusions can be drawn also for the second of the reaction types predominantly used in peptide tritiations, in the present work we investigated the catalytic deuteration of N-acetyl-DL-3,4-dehydroproline amide as a representative for multiple bond containing precursors. To facilitate a comparison between the results of the two labelling reactions, we applied the same catalysts and analogous reaction conditions as in the foregoing study.

#### Results and discussion

Regarding the distribution pattern in the molecular ion region of the mass spectra, clear differences can be seen in table 1 between the products obtained after reaction in dimethylacetamide (DMA) and after reaction in water. As indicated by the relative peak heights of the  ${}^2\text{H}_0$ - and  ${}^2\text{H}_1$ -species in the mass spectra, higher amounts of hydrogen were incorporated after reaction in water. Also higher after reaction in water is a deuterium incorporation preceding additionally to the saturation of the double bond into at least three positions of the molecule and up to an order of 25 percent of the total of deuterium incorporated, as can be derived from the  ${}^2\text{H}_3$  -  ${}^2\text{H}_5$ -species in the mass spectra (table 1). This additional deuterium must be incorporated before, or simultaneously with, the saturation of the double bond. So, if the saturation of the

Table 1: Mass spectrometric results after catalytic deuteration of N-acetyl-DL-3,4-dehydropoline amide using 96 % D<sub>2</sub> in the presence of Pd/Al<sub>2</sub>O<sub>3</sub> 10 %

Solvent	Pd/ sub- stra- te (mol/ mol)	I <sub>corr.</sub> m/z 100 <sup>a)</sup>						D/mol total	D/mol <sup>b)</sup> addi- tional	
		$\Sigma$ I <sub>corr.</sub> m/z	x=156 ( <sup>2</sup> H <sub>0</sub> )	x=157 ( <sup>2</sup> H <sub>1</sub> )	x=158 ( <sup>2</sup> H <sub>2</sub> )	x=159 ( <sup>2</sup> H <sub>3</sub> )	x=160 ( <sup>2</sup> H <sub>4</sub> )			x=161 ( <sup>2</sup> H <sub>5</sub> )
H <sub>2</sub> O	0.03	5.1	26.3	52.1	16.5	-	-	1.80	0.28	
"	0.16	5.8	26.3	50.1	17.2	0.6	-	1.81	0.31	
"	0.62	11.0	27.4	42.7	16.8	2.1	-	1.72	0.39	
"	1.56	17.8	27.9	38.5	13.3	2.2	0.4	1.56	0.36	
DMA	0.03	4.5	19.6	61.0	14.8	-	-	1.86	0.21	
"	0.16	4.3	21.3	60.5	13.5	0.4	-	1.84	0.20	
"	0.62	3.6	23.1	56.1	16.2	1.1	-	1.88	0.26	
"	1.56	4.6	22.8	51.3	18.1	2.6	0.5	1.93	0.34	
H <sub>2</sub> O <sup>c)</sup>	0.16 <sup>c)</sup>	12.6	30.3	38.0	14.3	3.9	0.8	1.69	0.48	
DMA <sup>c)</sup>	0.16 <sup>c)</sup>	2.4	20.9	60.1	14.2	1.7	0.7	1.94	0.24	
" <sup>d)</sup>	0.16	2.5	19.3	62.1	15.4	0.6	0.1	1.93	0.22	
DMA/H <sub>2</sub> O	96.5/23.5	0.16	2.5	22.5	60.0	14.8	-	-	1.87	0.22
"	"	1.56	10.3	27.2	43.2	15.4	3.3	0.7	1.77	0.39
DMA/Et <sub>3</sub> N (0.1 n)	0.16	1.2	15.8	72.3	10.6	-	-	1.92	0.13	
H <sub>2</sub> O/Et <sub>3</sub> N (0.1 n)	0.16	6.2	21.8	54.6	16.0	1.4	0.1	1.85	0.27	
H <sub>2</sub> O/HCl (0.1 n)	0.16	11.6	28.8	42.0	16.5	1.0	0.1	1.67	0.38	

- a)  $I_{\text{corr. m/z}}^x$  = 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 N-acetyl-DL-proline amide (M=156).
- b) Amount of deuterium incorporated additionally to the saturation of the double bond into the substrate (for calculation see Experimental).
- c) Reaction was performed in the presence of Rh/Al<sub>2</sub>O<sub>3</sub> 5% (0.16 mol rh/mol substrate)
- d) Reaction was performed at 40 °C.

double bond was incomplete, the reaction products showed in their mass spectra an enhanced peak at  $m/x$  155 ( $M+1$  of N-acetyldehydroproline amide), indicating a deuterium incorporation to occur before the saturation of the double bond. After the saturation of the double bond no deuterium incorporation could be detected as demonstrated by the treatment of N-acetylproline amide with  $D_2$  in  $D_2O$  (1,56 mol Pd/mol substrate; 1 h).

The amount of the additionally incorporated deuterium shows a similar dependence, not only on the kind of the solvent but also on the amount of the catalyst and on the pH, as the amount of hydrogen incorporated into the double bond [to be calculated by the difference:  $2 - (D/mol\ total - D/mol\ additional)$ ; table 1] and can compensate this hydrogen amount at least partially. Therefore the total deuterium amount of nearly two deuterons, which is to be expected after a simple saturation of a double bond, can be found in almost all products except those obtained in the presence of water at acidic pH or after use of high catalyst quantities.

When using other palladium catalysts comparable results were obtained. Analogously Hardy et al. /2/ found similar results after catalytic deuteration of N-acetyl-4,5-dehydro-DL-leucine with different palladium catalysts. Noticeable alterations could be observed only if a rhodium on-alumina catalyst was applied (table 1), which led in the dehalodeuterations to the highest hydrogen incorporations /1/. But also these alterations should not be principal ones because these results are comparable to those obtained with the fivefold amount of the palladium catalyst (table 1).

Therefore the results of table 1 allow the conclusion that neither a special catalyst preparation nor special reaction conditions are essential to achieve high labelling levels if dehydroproline is used as a precursor. The main practical conclusion is simply to minimize the quantity of a given catalyst to just such an amount which allows quantitative saturation of the double bond if solvents containing exchangeable hydrogen are used.

Principally the same could be concluded for the dehalodeuterations except that no influence of the pH on the unwanted hydrogen incorporation could be found there /1/.

At least twice the value for the isotopic effect  $K_H/K_D$  as found for the dehalodeuterations /1/ can be derived for the dehydroproline reaction from the experiments with different H/D-mixtures in table 2. This implies higher demands on the quality of the reaction gas for the labelling of dehydroproline containing precursors than for dehalogenations.

Table 2: Mass spectrometric results after catalytic deuteration (hydrogenation) of N-acetyl-DL-3,4-dehydroproline amide in the presence of Pd/AaI<sub>2</sub>O<sub>3</sub> 10% using different D<sub>2</sub>/H<sub>2</sub>-mixtures

Solvent	%H <sub>2</sub> in the D <sub>2</sub> -gas	mol Pd/mol substrate	$\frac{I_{\text{corr. m/z}} \times 100}{\sum I_{\text{corr. m/z}}}$ a)					D/mol total
			x=156 ( <sup>2</sup> H <sub>0</sub> )	x=157 ( <sup>2</sup> H <sub>1</sub> )	x=158 ( <sup>2</sup> H <sub>2</sub> )	x=159 ( <sup>2</sup> H <sub>3</sub> )	x=160 ( <sup>2</sup> H <sub>4</sub> )	
DMA	50	0.16	55.9	36.7	6.8	0.5	0.1	0.52
"	78	0.16	83.6	14.0	1.7	0.7	-	0.20
H <sub>2</sub> O	24	0.16	38.7	42.8	16.4	1.9	0.2	0.82
"	50	0.16	64.3	31.6	4.0	0.2	-	0.40
"	78	0.16	87.3	12.7	-	-	-	0.13
D <sub>2</sub> O	24	0.16	31.6	41.5	20.6	5.4	0.9	1.03
"	100	0.16	88.6	9.1	1.8	0.5	-	0.14
"	100	1.56	70.0	21.0	6.0	2.2	0.9	0.43

a) see footnote a) of table 1 (the relative intensity of the ms peak at m/z 161 was lower than 0.1 in all cases)

Based on a value of approximately 4 for the isotopic effect (derivable from table 2), an explanation of the amount of competing incorporated hydrogen of table 1 by the dilution of the reacting gas with solvent hydrogen would require gas-solvent exchanges in the order of 100  $\mu\text{mol}$  and higher within 10 minutes (see Experimental). But under analogous conditions in tritiation experiments only values in the order of 2-20  $\mu\text{mol/h}$  were found for the gas-solvent exchange /3,4/. Thus, to give a sufficient explanation for the degree of the found hydrogen incorporation, even a direct transfer of the solvent hydrogen to the substrate without a roundabout way across the gas phase should be taken into con-

sideration in agreement with findings of Marton and Kovacs /4/. Such a direct transfer could explain also the different distribution pattern in the mass spectra of reaction products obtained after deuteration in  $H_2O$  and hydrogenation in  $D_2O$  (table 2). If the deuterium would be incorporated after hydrogenation in  $D_2O$  only by a roundabout way across the gas phase, a similar distribution pattern as after deuteration in  $H_2O$  should be expected for products with comparable deuterium content.

### Experimental

N-acetyl-DL-3,4-dehydroproline amide was obtained by acetylation of DL-3,4-dehydroproline (obtained according to /5/ starting from pyrrole-2-carboxylic acid), followed by  $SOCl_2/CH_3OH$  - esterification and amidation in  $NH_3/CH_3OH$ .  $C_7H_{10}N_2O_2$ , m.p. 195-197 °C ( $H_2O$ ).

Palladium on alumina 10% and rhodium on alumina 5% were purchased from Fluka (Switzerland). Dimethylacetamide (Merck-Schuchardt, FRG) was distilled and stored successively over molecular sieve 10X and 3A before use.

The deuterium was produced by electrolyzing  $D_2O$  (98%), containing 5%  $H_2SO_4$ , on Pt-electrodes at a rate of about 100 ml/h and was conveyed directly through the reaction vessel.

To calculate the deuterium content in the gas, the isotopic effect  $K_H/K_D$  for the electrolysis was estimated to be 1.53 by measuring the gas volumes produced on the cathode after electrolyzing different  $D_2O/H_2O$ -mixtures (0.05 mol  $H_2SO_4$  and 5.3 mol  $H_2O + D_2O$  were mixed).

The deuteration was performed in a rectangular 20 ml Warburg-vessel, equipped with an additional side-bulb, under normal pressure and a constant flow of deuterium (100 ml/h) at ambient temperature. Normally 5 mg of N-acetyl-DL-3,4-dehydroproline amide, dissolved in 1.4 mol of the solvent and the catalyst were introduced separately into the reaction vessel and combined after 1h presaturation. After shaking for one hour (the saturation of the double bond was complete after 10 minutes except after reaction in DMA using lower mol Pd/mol substrate ratios than 0.1) the catalyst was removed by centrifugation and washed subsequently

with  $\text{CH}_3\text{OH}$  and  $\text{H}_2\text{O}$ . After evaporation of the combined solutions the remaining residue was dissolved in  $\text{CH}_3\text{OH}/\text{H}_2\text{O}$  and reevaporated twice, and was then used for mass spectrometric measurements without further purification.

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  $m/z$  156 - 161, corrected for the natural isotope abundance, estimated in the mass spectrum of N-acetyl-DL-proline amide (obtained by hydrogenation of the dehydroproline derivative). To warrant a reproducible precision of the results, 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 three recordings of the same sample.

The amount of deuterium incorporated additionally to the saturation of the double bond was calculated by the following relations obtained under the simplified assumptions that the saturation of the double bond and the incorporation of the additional deuterium proceed independently of each other and by neglecting the species containing more than one additional deuterium:

$$A - D \cdot A = I_0; B + D \cdot A - D \cdot B = I_1;$$

$$C + D \cdot B - D \cdot C = I_2; D \cdot C = I_3.$$

( $I_x$  = relative intensities of the peaks at  $m/z$  156 - 161 of the mass spectra given in table 1, divided by 100.

$x = 0$ :  $m/z = 156$ ;  $x = 1$ :  $m/z = 157$ ;  $x = 2$ :  $m/z = 158$ ;

$x = 3$ :  $m/z = \sum 159-161$ .

A, B, C = relative intensities of the peaks at  $m/z$  156, 157, 158 (divided by 100) in the absence of additionally incorporated deuterium.

D = amount of deuterium incorporated additionally to the saturation of the double bond.)

The equation resulting from these relations was cardanic for all values given in table 1 and in all cases there exists only one real solution:

$$D^3 - (I_1 + 2 \cdot I_2 + 3 \cdot I_3) \cdot D^2 + (I_2 + 3 \cdot I_3) \cdot D - I_3 = 0.$$

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