

CATALYTIC DEHALOGENATION OF N-ACETYL-L-4-CHLORO- AND N-ACETYL-L-4-IODOPHENYLALANINE AMIDE IN THE PRESENCE OF DEUTERIUM

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Summary

As a model for the tritium labeling of peptides, the catalytic dehalogenation of N-Acetyl-L-4-chloro- and N-Acetyl-L-4-iodo-phenylalanine amide was investigated in the presence of deuterium, using different reaction conditions. A catalyst-mediated transfer of the solvent-hydrogen to the substrate was found to be the most probable reason for the exchange of halogen by hydrogen instead of deuterium. This unwanted transfer was most intensive in the presence of water. An incorporation of additional deuterium besides the 4-position of phenylalanine takes place simultaneously with the dehalogenation especially of the chloro derivative.

Key words: Deuterium gas, catalytical dehalogenation, solvent-substrate - hydrogen transfer

Introduction

The catalytic dehalogenation of halogen derivatives in the presence of tritium gas in one of the most widespread methods for

tritium labeling. Despite numerous experiments in using this method the theoretically expected specific activities are rarely attained. A decrease of the specific activity of the tritium gas, caused by a rapid catalyst-mediated exchange with solvent-hydrogens is often discussed as a reason for the unsatisfactory tritiation yields. There exist also indications for a direct hydrogen transfer reaction between the solvent and the substrate, occurring on the surface of the catalyst /1,2/.

To improve our understanding of the influence of different reaction conditions on the labeling result we investigated in model reactions the catalytic dehalogenation by deuterium gas of N-Acetyl-4-chloro- (I) and -iodophenylalanine amide (II) and calculated the amount of incorporated deuterium on the basis of ir and ms measurements.

Experimental

N-Acetyl-L-4-chlorophenylalanine amide (I) was obtained by $\text{SOCl}_2/\text{CH}_3\text{OH}$ -esterification of N-Acetyl-L-4-chlorophenylalanine, amidation in $\text{NH}_3/\text{CH}_3\text{OH}$ and recrystallisation from 1 n NH_3 .

$\text{C}_{11}\text{H}_{13}\text{ClN}_2\text{O}_2$, m.p. 235 °C, $[\alpha]_D^{20} = 33.1$ (c = 1, CH_3OH).

N-Acetyl-L-4-iodophenylalanine amide (II), $\text{C}_{11}\text{H}_{13}\text{IN}_2\text{O}_2$, m.p. 210 °C and N-Acetyl-L-phenylalanine amide (III), $\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_2$, m.p. 180 °C, $[\alpha]_D^{20} = 48.0$ (c = 1, CH_3OH) were obtained under analogous conditions.

The deuteration was performed in rectangular 20-ml-Warburg-vessels, equipped with an additional side-bulb, under normal pressure and a constant flow of deuterium (60 ml/h). The deuterium was produced by electrolyzing D_2O (98 %), containing 5 % H_2SO_4 , on Pt-electrodes at a rate of about 60 ml/h and was conveyed directly through the reaction vessel.

Normally the catalyst, suspended in 1 ml solvent (distilled and

dried over molecular sieve 10X or 3 A) and 9 μ mol substrate, dissolved in 0.4 ml solvent, were introduced separately into the reaction vessel and combined after 1 h presaturation under shaking. After a reaction time of one hour under shaking the catalyst was removed by centrifugation and washed subsequently with CH₃OH and H₂O. After evaporation of the combined solutions the remaining residue was dissolved in CH₃OH/H₂O and reevaporated twice, and was then used without further purification for ir- and ms-measurements.

Hydrogenations were performed analogously.

Mass spectra were recorded on a MS 902 S spectrometer (AEI, Manchester, England). The degree of deuteration was determined by means of the intensities of the peaks m/z 206 - 209, corrected for the natural isotope abundance, estimated in the mass spectrum of III. The intensities were determined as an average of five recordings of the same sample.

Ir-spectra were recorded on a Specord 75 IR spectrometer (VEB Carl Zeiss Jena) using KBr-pellets.

The ir spectra of the hydrodehalogenation-products of I and II were identical with the ir spectrum of III. After deuteration the relative intensities (related to the intensity of the amide band at 1630 cm^{-1} , remaining unchanged) of ir peaks at 608 and 697 cm^{-1} showed a good correlation to the deuterium content of the products, determined by ms (fig. 1) and allowed a quantitative estimation of the degree of deuteration.

In fig. 1 the increasing D-peak at 608 cm^{-1} and the decreasing H band at 697 cm^{-1} have nearly the same molar extinctions, and the sum of their relative extinctions in mixtures of the deuterated and the nondeuterated samples is nearly constant. This allows to use the quotient $E_{697}/E_{608} + E_{697}$ (all corrected by the absorptions of the background) for the esti-

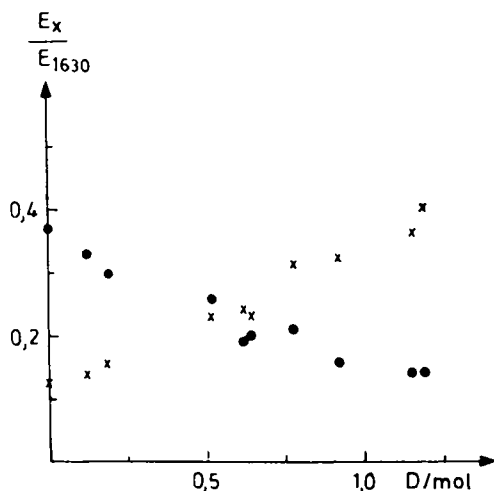


Fig. 1. Dependence of the relative intensities (extinction at $x \text{ cm}^{-1}$ /extinction at 1630 cm^{-1}) of the ir-bands at $x = 697 \text{ cm}^{-1}$ (...) and $x = 608 \text{ cm}^{-1}$ (xxx) from the deuterium content (determined by means of ms) of III, obtained by catalytic deuteration of I (5 mg I; 5 mg $\text{Pd}/\text{Al}_2\text{O}_3$ 10 %; 1.4 ml $\text{H}_2\text{O}(\text{D}_2\text{O})/\text{CH}_3\text{COOH}$ 1.1/0.3; D_2H_2 -mixtures containing 0-95 % D_2).

mation of the hydrogen incorporation competing with the deuteration. This method allowed a sufficient estimation even when the dehalogenation was incomplete and in the presence of traces of the solvent in contrast to the use of E_{697}/E_{1630} (fig. 2).

Results and discussion

In aqueous solutions the dehalogenation of I and II was quantitative within one hour, independently of the carrier of the catalyst (carbon, Al_2O_3 , BaSO_4 , CaCO_3), when a ratio of substrate to catalyst-metal (Pd, Rh) of 5 to 1 was used. In dimethylacetamide (DMA) only a partial dehalogenation of 10 to 50 percent took place when using this substrate-catalyst ratio, even after several hours. Taking the fivefold excess of the catalyst or addition of triethylamine gave quantitative dehalogenation within one hour also in the aprotic solvent. Despite the complete dehalogenation the amount of incorporated

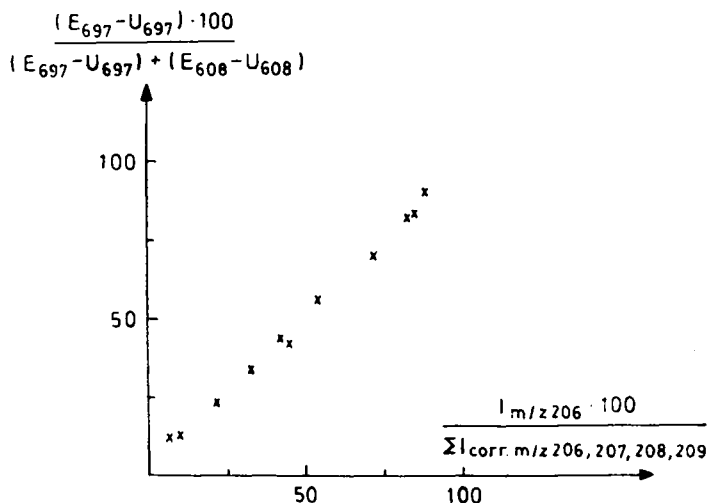


Fig. 2. Dependence of the quotient from the extinction of the ir-band at 697 cm^{-1} (E_{697}), corrected by the extinction of the background (U_{697}) and the sum of the extinctions of the ir-bands at 697 and 608 cm^{-1} (corrected by the extinctions of the background) from the amount of nondeuterated III in the dehalogenated reaction product, obtained after catalytic deuteration of I or II ($I_{m/z\ x}$ = intensity of the ms peak at the mass number x ; $I_{\text{corr.}}$ = see table 5).

deuterium differed considerably. A strong influence of the solvent, the catalyst and the substrate-to-catalyst ratio was found (table 1).

The amount of hydrogen found in III instead of deuterium was higher when the dehalogenation was carried out in water containing solutions than in the absence of water (tables 1 and 2). Interestingly the maximum amount of incorporated hydrogen was reached already in the presence of 3 percent water and more water did not increase the hydrogen content of deuterated products. The data in tables 1 and 2 demonstrate, moreover, that the competing H-incorporation can be diminished by lowering the catalyst-to-substrate ratio. This observation is supported

by the finding of Lebl et al. /3/, that, on tritiodahalogenation of diiodotyrosine-oxytocine, lower amounts of catalyst give higher specific activities.

Table 1: The amount of nondeuterated III obtained after deuteration of I and II as a function of reaction conditions

Substrate	Solvent	Catalyst	mol substrate/ mol Pd or Rh	T (°C)	%III ^{a)}
I	DMA	Pd/Al ₂ O ₃ 10%	0.9	20	19
"	DMA/H ₂ O 97/3 ^c	"	"	"	37
"	DMA/H ₂ O 50/50	"	"	"	37
II	DMA	"	"	"	15
"	DMA/H ₂ O 97/3 ^c	"	"	"	25
"	DMA/H ₂ O 50/50	"	"	"	28
"	"	"	"	60	31
II	DMA/H ₂ O 50/50	Pd/Al ₂ O ₃ 10%	0.24	20	47
"	DMA	Pd/C 10%	0.9	"	13
"	DMA/H ₂ O 50/50	"	"	"	34
"	DMA	Rh/Al ₂ O ₃ 5%	"	"	41
"	DMA/H ₂ O 97/3 ^c	"	"	"	76
"	DMA/H ₂ O 50/50	"	"	"	70

a) %III = amount of nondeuterated III in percent of the dehalogenated reaction product obtained after catalytical deuteration of I or II, calculated from the mass-spectra or the ir-spectra (see fig. 2).

Table 1 demonstrates also striking differences between palladium and rhodium. The experiments with rhodium are characterized by

Table 2: Amount of nondeuterated III after deuteration (hydrogenation) of I in H₂O and D₂O

Solvent	Reacting gas	mol I/mol Pd (Pd/Al ₂ O ₃ 10%)	%III
D ₂ O/CH ₃ COOH 1.1/0.3	H ₂	4.4	85 (±0.17 D/mol)
"	D ₂	"	7
"	"	2.2	10
H ₂ O/CH ₃ COOH 1.1/0.3	"	4.4	22
"	"	2.2	33

a remarkably higher H-incorporation into III even when water was removed carefully.

We could not find any influence of temperature on the ratio of H/D-incorporation in the dehalogenation products (table 1).

The nature of the halogen does not seem to influence markedly the H/D-ratio. The chlorine derivative I leads to a slightly higher rate of incorporated hydrogen than the iodine derivative II (table 1).

Surprisingly we could not find any influence of pH on the incorporation of hydrogen (table 3). It is remarkable that in all experiments (table 3) quantitative dehalogenation was found, even in the presence of hydrochloric acid which was believed to act as a poison for catalysts.

The results discussed so far raise the question on the origin and the mechanism of the hydrogen incorporation during the deuterodehalogenation.

In addition to water, one may discuss as a source of hydrogen the aprotic solvent, the substrate itself, especially the amide protons, and traces of water adsorbed onto the catalyst. However the pretreatment of catalyst and substrate with D₂O

Table 3: Amount of nondeuterated III after deuteration of I.
In all cases the molar ratio I/Pd = 4.4 and the dehalogenation was complete.

Solvent	Catalyst	%III
CH ₃ OH/H ₂ O 0.7/0.7	Pd/Al ₂ O ₃ 10 %	20
CH ₃ OH/H ₂ O/(C ₂ H ₅) ₃ N 0.7/0.6/0.1	"	20
CH ₃ OH/H ₂ O/25% NH ₃ 0.7/0.6/0.1	"	26
CH ₃ OH/H ₂ O/CH ₃ COOH 0.7/0.6/0.1	"	20
CH ₃ OH/H ₂ O 0.7/0.7	Pd/C 5%	25
CH ₃ OH/H ₂ O/(C ₂ H ₅) ₃ N 0.7/0.6/0.1	"	27
CH ₃ OH/H ₂ O/1n HCl 0.7/0.6/0.1	"	25

followed by drying has no influence on the rate of hydrogen incorporation in DMA. Thus, only the solvent seems to be significantly involved in a catalyst-mediated transfer of hydrogen into the 4-position of I and II.

If a catalyst-mediated gas-solvent exchange would be responsible for the observed hydrogen-incorporation, such a gas-solvent exchange should have a rate of at least 0.5 mmol/h under the reaction conditions used here (open system, 20 ml gas volume, 60 ml/h D₂-flow; see experimental part) and taking into account an isotopic effect of maximally 3 (deducible from table 4). However the slight rise of 3 - 5 percent hydrogen incorporation which was found after reaction with a constant D-amount in the closed reaction vessel corresponds with a gas-solvent exchange in a range of only 3 - 10 μmol/h (DMA/H₂O 1/1; II/Pd 0.9/1 (Pd/Al₂O₃

Table 4: Amount of nondeuterated III obtained after deuteration of I in the presence of Pd/Al₂O₃ 10% (mol I/mol Pd = 4.4) using D₂/H₂ - mixtures produced by electrolyzing D₂O/H₂O mixtures (containing 5% H₂SO₄).

Solvent		%D ₂ O in the electrolytic mixture	%III
D ₂ O/CH ₃ COOH	1.1/0.3	98	7
H ₂ O/ "	" "	"	22
D ₂ O/ "	" "	60	54
H ₂ O/ "	" "	"	72
D ₂ O/ "	" "	30	82
H ₂ O/ "	" "	"	90

10%); 1.5 h presaturation with open system and flowing D₂, then closing the reaction vessel, stopping the production of D₂ and, after 30 min. further presaturation, combining substrate and catalyst and 1 h deuteration). This is in agreement with rates of catalyst-mediated tritium-solvent exchange, found by Černý and Hanuš /4,5/ using comparable catalysts and catalyst-solvent ratios as used here. Such a low rate of gas-solvent exchange cannot be the reason of the observed hydrogen incorporation. Therefore the solvent-hydrogen should be transferred by another mechanism directly to the substrate.

In contrast to the undesired transfer of hydrogen the amount of incorporated deuterium could increase when besides the halogen-deuterium exchange a nonspecific H-D exchange in positions other than in 4 takes place, as described for phenylalanine /2,6/. Indeed, analyzing the mass spectra of samples with a high degree of deuteration, we found considerable intensities of M+2 and even of the M+3 peaks amounting to an additional D-incorporation up to 0.25 D/mol. Correlated to the M+2 peak, another peak shifted from m/z 77 to m/z 79 could be observed, indicating a D-incorporation into the phenyl ring. This was supported by the observation that the additional D-incorpora-

tion seems to have the same effect on the ir spectrum as the D-incorporation in position 4. No other ir bands could be found and the intensities of the ir peaks at 697 and 608 cm^{-1} fit well with the ms data without any dependence on the amount of additionally bound deuterium in the sample (fig. 2). An explanation for the contradiction between the incorporation in the phenyl ring, found here, and the substitution of benzylic hydrogens, found by direct tritium labeling of phenylalanine /2,6/, could be given by the observation that the main part of the additional deuterium incorporation into I and II depends on the presence of aromatically bound halogen and therefore could be incorporated by an alternative mechanism. Under those reaction conditions leading to 0.25 additional D/mol into I the nonhalogen-containing compound III incorporates only 0.04 D/mol. On the other hand, in experiments with incomplete dehalogenation the deuterium was only found in the ms peaks of the dehalogenated products and not in the ms peaks of the remaining halogen derivatives. These observations lead to the conclusion, that the nonspecific, additional D-incorporation takes place simultaneously with the dehalogenation step. In table 5 the influence of solvent, catalyst and halogen on the degree of additional D-incorporation is summarized. The content of deuterium in III has been found to be higher in aqueous solutions than in DMA, higher in the presence of rhodium than in the presence of palladium, and higher after deuteration of the chloro-compound I as compared to the iodine-derivative II.

Conclusion

A main reason for the incorporation of hydrogen during deutero-dehalogenation is a direct catalyst-mediated transfer of solvent-hydrogen, especially from water but also from aprotic solvents, to the substrate. The effect of such a side reaction in addition

Table 5: Dependence of the unspecific additional deuterium incorporation, indicated by the ms peaks at m/z 208 and 209 (${}^2\text{H}_2$ and ${}^2\text{H}_3$) versus the reaction conditions

Substrate	Solvent	Catalyst	mol substrate/ mol Pd or Rh	$I_{\text{corr.}m/z} \cdot x \cdot 100$			
				$I_{\text{corr.}m/z}$ 206,207,208,209			
				$x=206$ (${}^2\text{H}_0$)	$x=207$ (${}^2\text{H}_1$)	$x=208$ (${}^2\text{H}_2$)	$x=209$ (${}^2\text{H}_3$)
I	$\text{H}_2\text{O}/$ CH_3COOH 1.7/0.3	$\text{Pd}/\text{Al}_2\text{O}_3$ 10%	4.4	21.6	66.0	10.5	1.8
I	"	"	2.2	32.5	58.6	7.3	1.5
I	DMA	"	0.9	19.6	75.0	5.4	-
I	"	$\text{Rh}/\text{Al}_2\text{O}_3$ 5%	4.4	47.1	35.4	12.7	4.8
II	"	$\text{Pd}/\text{Al}_2\text{O}_3$ 10%	0.9	15.1	83.5	1.4	-
II	"	$\text{Rh}/\text{Al}_2\text{O}_3$ 5%	"	40.6	57.4	2.0	-

a) $I_{\text{corr.}m/z} \cdot x$ = Intensity of the ms peak appearing at the mentioned mass number diminished by the intensity of the natural isotopic peak related to $x - 1$, which was estimated by means of the mass-spectrum of nondeuterated III ($M=206$).

to the effects of impurities in the reacting gas and of the catalyst-mediated direct solvent-gas exchange should be sufficient to explain the low specific activities, often found in tritiation experiments. On the other hand, on the basis of these results it seems possible, generally, to achieve specific activities higher than 70% of the theoretical ones in triodehalogenations by optimizing the choice of the solvent and of the nature and amount of catalyst. The prediction of optimal conditions for a given problem may be facilitated by testing catalysts and solvents before the tritiation, using an analogous model reaction as described here.

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