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EFFECTS OF CATALYST PRETREATMENT AND ADDITIVES ON THE HYDROGEN INCORPORATION DURING THE CATALYTIC DEUTERATION OF N-ACETYL-DI-IODOTYROSINEAMIDE

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Summary

The influence of the mode of presaturating the catalyst and of different inhibiting agents on the catalytic deuteration of N-acetyl-3,5-di-iodo-L-tyrosineamide was investigated in order to derive conclusions for optimizing peptide tritiations. Reactions of the activated catalyst with solvent and substrate can be inhibited independently. The exchange of the catalyst-bound deuterium against hydrogen from solvent is inhibited by the substrate itself and by addition of 1,3-di-chlorobenzene. The dehalogenation reaction and the unspecific exchange of carbon-bound hydrogen of the substrate are inhibited preferably by thioglycolic acid, iodide and by the triethylammonium ion.

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

Introduction

A general drawback of catalytic tritiations is the unreliability of the labelling results, mainly caused by unwanted incorporation of hydrogen /1/. To obtain more reliable labelling results, a better understanding of basic reactions proceeding during a catalytic tritiation is needed. To contribute to this aim, we investigated in preceding studies the catalytic deuteration of derivatives of 4-halogenated phenylalanines, dehydroproline and di-iodotyrosine /2,3,4/.

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We found simple relations between the deuteration results and the type of solvent and catalyst used and the catalyst-to-substrate ratio /2,3,4/, which have been proved to be valid also for catalytic tritiations for peptides /5,6/. Furthermore we could show that the major part of the solvent hydrogen incorporated into the substrate was introduced in a direct way, independently of a dilution of the reacting gas /2,4/.

In order to obtain more detailed information on the mode of incorporation of solvent hydrogen, the influence of the catalyst-to-substrate ratio and the role of iodide generated during the reaction, in the present study we have investigated the catalytic deuteration of N-acetyl-3,5-di-iodo-L-tyrosineamide ($AcDitNH_2$) after different pretreatments of the catalyst and with addition of several inhibiting agents.

Experimental

N-Acetyl-3,5-di-iodo-L-tyrosineamide ($AcDitNH_2$), Dimethyl-acetamide (DMA), deuterated DMA and the catalyst (Pd/Al_2O_3 10%) were of the same batch as used in the earlier study /4/. Triethylamine (VEB Laborchemie Apolda, GDR) was distilled and stored over sodium.

The deuterations (4.5 mg $AcDitNH_2$, 1.4 ml DMA/H_2O 1/1, 50 mg Pd/Al_2O_3 10 % presaturated in dry form, 20 ml gasvolume, flowing D_2, 100 ml D_2/h) were performed as described earlier /4/ (one hour presaturation followed by one hour deuteration under shaking). The dehalogenation was complete in all cases except the reaction in the presence of 10 µmol thioglycolic acid (about 70% dehalogenation).

The deuterium content in the dehalogenation product was determined mass spectrometrically as described previously /4/ using the peaks m/z 222-226 (M^+ of deuterated N-acetyl-tyrosineamide). Repetition of the deuteration experiments gave no deviations in the deuterium content of the reaction product higher than 0.05 D/mol.

In distinction from the earlier study /4/, the deuterated reaction product was purified by tlc on silica gel F254 plates (Merck, FRG) before the mass spectrometry, using $CHCl_3/CH_3OH/CH_3COOH$ 15/1/1. The uv-detected spots were scraped out, and the substance was extracted with methanol. During tlc no loss of label takes place as a comparison of the results of deuteration experiments without additives obtained before and after tlc purification showed.

Results and dicussion

The degree of deuteration in the dehalogenated product of the catalytic deuteration of $AcDitNH_2$ shows a strong dependence on the mode of pretreating the catalyst (table). This underlines the role of the operation mode for the labelling practice.

The results fit well the assumption of independent reactions of the deuterated catalyst with both the solvent and the halogenated substrate competing with each other. Likewise the results show that reactions of intermediate substrate species or catalyst-substrate complexes with the solvent cannot have noticeable importance for the rate of incorporation of ^1H into the reaction product (to be taken from the difference: 2- D/mol). Especially the remarkably higher incorporation of ^1H after presaturating the catalyst in the solvent compared to the results of the other two operation modes (table) would be in contradiction to an explanation by such reactions.

As demonstrated previously /2,4/, the major part of the ^1H incorporated under the reaction conditions used here (see Experimental) is transferred from the solvent directly to the substrate independently of a dilution of the reacting gas. Thus the observed results have to be interpreted without taking into account a different enrichment of the reacting gas with ^1H. Such interpretation presupposes that the halogenated substrate must be able to react more rapidly with the activated catalyst than the exchange of the catalyst bound hydrogen isotope against the reacting gas proceeds. This was proved by the following experiments:

The catalyst was presaturated in DMA/D_2O 1/1 with hydrogen under shaking for one hour. After 90 minutes exchange of the hydrogen by deuterium without shaking (D_2 flow 100 ml/h) $AcDitNH_2$ was added and the mixture was shaken for

Table: Mass spectrometrically detected deuterium contents in the dehalogenated products obtained after 1h catalytic deuteration of $AcDitNH_2$ (4.5 mg) in DMA/H_2O 1/1 in the presence of Pd/Al_2O_3 10% (50 mg) using different catalyst pretreatment and inhibiting additives

Mode of operation x)	Additive	deuterium content (D/mol)
A	140 µmol Et_3N	0.89
B	140 µmol Et_3N	1.72
C	140 µmol Et_3N	1.54
C xx)	140 µmol Et_3N	1.75
C	140 µmol Et_3N + 20 µmol 1,3-dichlorobenzene	1.62
C	280 µmol Et_3N + 100 µmol 1,3-dichlorobenzene	1.68
C	-	1.57
C	1 µmol thioglycolic acic	1.24
C	10 µmol thioglycolic acid	0.60
C	120 µmol KJ	1.36
C	120 µmol $Et_3N \cdot HCl$	1.36

x) A: Catalyst was presaturated by shaking under D_2 for 1 hour in the solvent before addition of $AcDitNH_2$

B: Reaction was performed without separate presaturating the catalyst by shaking the combined reaction mixture under D_2 for 1 hour

C: Catalyst was presaturated in the dry form with D_2 for 1 hour before combining with the solution of $AcDitNH_2$ (see Experimental)

xx) Reaction was performed in the presence of 10 mg Pd/Al_2O_3 10 %

30 minutes. An ^1H incorporation of 0.55 H/mol was found. The same experiment was repeated with shaking the catalyst suspension under deuterium for 2, 10 and 20 minutes before the addition of the substrate. In all cases the clearly lower ^1H incorporation of 0.35 H/mol (due to the remaining hydrogen content in the gas) was found. Thus in the first case the substrate must

have incorporated some of the hydrogen remaining on the catalyst more quickly than the exchange of the catalyst-bound hydrogen with the reacting gas proceeds. In a reversed experiment using deuterium for the presaturation and hydrogen for the dehalogenation in DMA/D_2O analogously 0.63 D/mol (without shaking) and 0.38 D/mol (due to the deuterium content in the gas and to a deuterium transfer from D_2O) after preshaking the catalyst were obtained.

Without presaturating the catalyst a clearly higher deuteration degree was obtained than after separate presaturating the catalyst before the reaction (table), although about 30 min were needed in this case to achieve a complete dehalogenation compared to about 5 - 10 min after presaturating the catalyst. The comparison of the results from using the dryly presaturated catalyst and from reaction without presaturating of the catalyst (table) leads us to the conclusion that the halogenated substrate competes not only with the solvent for the activated catalyst but also inhibits the H-exchange with the solvent. Such an inhibiting effect being more intensive at higher substrate-to-catalyst ratios could be a reason for the generally observed influence of the substrate-to-catalyst ratio on the incorporation of solvent hydrogen into the substrate /2-6/ (table).

On the other hand, a partial poisoning of the catalyst by thioglycolic acid led to an enhanced incorporation of solvent hydrogen into the substrate indicating a more intensive inhibition of the substrate-catalyst reaction than of the solvent-catalyst reaction (table). A special influence of the reagent on radicalic or ionic reactions of the activated catalyst could not be detected. Both the transfer of hydrogen from water and from DMA to the substrate were enhanced about twofold by the addition of 10 µmol thioglycolic acid, as shown by experiments using tritiated water and deuterated DMA in the solvent according to /4/.

The incorporation of ^1H into the substrate was enhanced also in the presence of iodide and the triethylammonium ion (table). The presence of NaF, KCl, KBr, LiCl, CsCl, NH_4Cl, $KOOCCH_3$ (120 µmol), K_2HPO_4, $Na_2B_4O_7$ (60 µmol) or of pyridine (120 and 1200 µmol) or pyridinehydrochloride (120 µmol) did not

affect the deuteration result (outside of the measuring error).
However CERNY and HANUS found remarkable effects of different buffer salts on the catalyst-mediated tritium exchange into solvents in the absence of a substrate /7/. Thus it seems likely that the effects of some of the salts used here on the catalyst-solvent reaction will be compensated by similiar effects on the catalyst-substrate reaction.
On the other hand the effect of hydrochloric acid observed in the previous study /4/ (DMA/0.2 m HCl 1/1, otherwise the same conditions as used here; result: 1.36 D/mol) should be caused by a promotion of the catalyst-solvent reaction by H^+ rather than by an inhibition of the catalyst-substrate reaction, taking into account the lack of influence of chloride and different alkali metal ions observed here. This interpretation consists with the suggestion of ANWER et al. /8/ that the catalyst is poisoned preferably by the nondissociated hydrogen halide during a hydrogenolysis of aryl halides and not by the halide anion present in a polar or basic medium. Own earlier observations concerning the dehalogenation reactivity in dry and water containing DMA in the presence or absence of triethylamine /2,4/ are in accordance with this suggestion too.

Whereas the influence of iodide on the dehalogenation reaction is relatively low (table) and could not be detected after addition of only 20 μmol KJ, its influence on the additional introduction of label into the benzylic positions according to EVANS et al. /9/ should be of importance also for the labelling practice. The presence of 20 μmol KJ lead to an almost complete suppression of a deuterium incorporation into N-acetyl-tyrosineamide during a catalytic deuteration both in DMA/H_2O 1/1 and DMA/D_2O 1/1. In the absence of iodide, 0.5 and 0.9 D/mol respectively were incorporated under the same conditions /4/. Thus the iodide generated during the dehalogenation of Ac-DitNH$_2$ seems to be responsible for the generally low introduction of additional label of below 0.1 D/mol (derived from the $^2H_3$- and $^2H_4$-species in the mass spectra) found here and earlier /4/. The same explanation should be valid for the previous finding that a prolonged reaction time did not enhance the additional labelling during the dehalodeuteration of $AcDitNH_2$ /4/.

Conclusions

The hydrogen incorporation during the catalytic dehalodeuteration of $AcDitNH_2$ is caused predominantly by a reaction of the activated catalyst with the solvent proceeding before the reaction with the substrate. The reaction with the substrate competes successfully with the exchange of the catalyst-bound solvent-hydrogen into the reacting gas.

The interaction of the activated catalyst with the solvent is decreased by the halogenated substrate, whereas the reaction with both the halogenated and the dehalogenated substrate is inhibited by the iodide ion generated from $AcDitNH_2$. The results allow the conclusion that the influence of buffer salts and of impurities which have to be expected in labelling samples of a few micromoles should be of clearly less importance for labelling results than the type of catalyst and solvent, the catalyst-to-substrate ratio /2-6/ and the mode of pretreating the catalyst.

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