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Heterogeneous Platinum-Catalyzed Deuterium Exchange of Aromatic Protons in Amino Acids, Peptides and Proteins

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In an attempt to simplify the proton magnetic resonance (PMR) spectra of proteins by exchange of amino acid side chain protons with deuterium, the platinum-catalyzed exchange of the aromatic protons of L-phenylalanine, a tripeptide containing phenylalanine and two proteins have been studied using PMR spectroscopy. Deuteration of the small model compounds proceed satisfactorily under mild conditions, but no exchange was observed of the aromatic protons of phenylalanyl or tyrosyl residues of native or denatured lysozyme or ribonuclease. Several possible explanations are suggested for this lack of exchange.

INTRODUCTION

The proton magnetic resonance spectra of native proteins generally consist of broad envelopes because of overlap of the resonances of different types of amino acids, chemical shift nonequivalence among resonances from a single type of amino acid, and broadening of individual resonances due to the slow molecular motion of proteins in solution (*1*). In order to resolve individual proton resonances from these broad envelopes, a number of methods have been developed (*2–6*). One of these has been to prepare proteins in which all but a few of the amino acid residues have been deuterated, by growing a microorganism on a defined mixture of protonated and deuterated amino acids (*5,6*). Unfortunately, this technique is time-consuming and expensive and is limited to those proteins which can be isolated from microorganisms. A more general approach would involve the selective deuteration of residues in intact proteins and the use of PMR difference spectroscopy (*2,7*) or deuterium magnetic resonance spectroscopy to observe the deuterated residues. Thus we have further developed methods for the selective deuteration of histidine (*8–10*) and tryptophan (*11–13*) residues in proteins and report here the results of our attempts to use heterogeneous platinum catalysts for deuterium exchange of proteins.

The platinum-catalyzed exchange of the aromatic protons of various organic compounds, including amino acids and peptides, has been studied extensively (*14,15*). Reduced platinum(II) oxide catalyst has been used in our work because it was found by previous workers (*15,16*) to be the most active of the various metal oxides and chlorides studied.

METHODS

Catalyst was prepared by reduction with hydrogen gas in a two-step procedure which involves prereduction and then an activation step just prior to use (*17*) (this preparation is designated "A") or by reduction with potassium borohydride (*15,16*) to produce reduced catalyst designated "B". Exchange was carried out by

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placing the ampoule of activated platinum catalyst (A) or the reduced catalyst (B) in the reaction vessel, followed by the substrate solution in D_2O. Oxygen was removed by four to five freeze–pump–thaw cycles and the reaction vessel sealed *in vacuo*. In the case of the catalyst A the ampoule was then broken in the reaction vessel and reaction proceeded under various conditions (see Tables 1–3). Afterwards, the vessel was opened and the reaction mixture centrifuged to remove catalyst.

The extent of deuterium incorporation was determined by PMR spectroscopy using a Jeol MH-100 PMR spectrometer. For the amino acids and peptides, exchange is expressed as the percentage by which the ratio of the area (or height) of the aromatic proton resonance to the area (or height) of a nonexchanging aliphatic proton resonance has decreased in the exchanged sample relative to an untreated sample. For proteins, exchange was determined by subtracting the PMR spectrum of the exchanged protein from the spectrum of the untreated protein and comparing the area of any difference peak with that of a nonexchanged resonance of known area present in the spectrum of the untreated protein.

RESULTS AND DISCUSSION

The results for the deuterium exchange of the aromatic protons of L-phenylalanine and L-leucyl-glycyl-L-phenylalanine using catalysts A and B are given in Tables 1 and 2, respectively. The activity of catalyst A falls off slowly with the time which elapses between the prereduction of the platinum oxide and its activation (Table 1), whereas catalyst B is quite stable for up to 6 mo after preparation (Table 2). The activity of catalyst A towards L-phenylalanine is marginally greater than that of catalyst B but the latter is easier to prepare and use. The activities of both catalysts are increased by increase of temperature (with partial exchange of aliphatic protons at 109°C), but decreased markedly by the presence of deuterated guanidine hydrochloride (6.5 *M*), which causes unfolding of most proteins (*7,18,19*).

TABLE 1
EXCHANGE OF L-PHENYLALANINE IN D_2O USING CATALYST A[a]

Time lapse (days) between prereduction and activation	Conditions of exchange		Exchange of aromatic protons (%)
	Temp (°C)	Time (days)	
8	22	6	68
8	37	4.3	86
10	50	3	78
10	75	2	93
29	37	4.25	50
55[b]	37	4.17	0
169	37	4.25	8

[a] L-Phenylalanine (50 mg) was dissolved in 2 ml D_2O (99.8%, Australian Atomic Energy Commission) and treated with 5 mg of activated catalyst A.

[b] Solution contained 6.5 *M* deuterated guanidine hydrochloride (GuDCl), which was obtained by lyophilization of guanidine hydrochloride three times from D_2O.

TABLE 2
EXCHANGE OF L-PHENYLALANINE AND L-LEUCYL-GLYCYL-L-PHENYLALANINE USING CATALYST B[a]

Substrate	Solvent	Conditions of exchange		Exchange of aromatic protons (%)
		Temp (°C)	Time (days)	
L-Phenylalanine	D_2O, pH 7	109	1	100[b]
L-Phenylalanine	D_2O	50	3	71
L-Phenylalanine	D_2O, pH 6	37	5	67
L-Phenylalanine	6.5 *M* GuDCl	50	3	12
L-Phenylalanine	6.5 *M* GuDCl	37	5	19
Leu-Gly-Phe	D_2O, pH 2.6	70	1	62
Leu-Gly-Phe	6.5 *M* GuDCl	50	2	0
Leu-Gly-Phe	6.5 *M* GuDCl	37	5	0

[a] Substrate (10 mg) was dissolved in 1 ml D_2O (99.8%) and exchanged using 10 mg of catalyst B. The catalyst was always used within 1–2 wk of preparation although its activity towards L-phenylalanine did not change after 6 mo storage.

[b] At 109°C there was partial exchange of α and β protons; no exchange of aliphatic protons was observed in any other experiment at temperatures ≤75°C.

Attempts were made to exchange the aromatic protons of lysozyme and ribonuclease using the conditions given in Table 3. After the treatments the catalyst was removed by centrifugation and the solution was lyophilized. In the case of the GuDCl treatment the latter was removed by dialysis against distilled water for 3 days before lyophilization. The degree of deuterium exchange was determined by examining the PMR spectrum of a 10% solution of the protein in 8 *M* urea containing 0.2 *M* 2-mercaptoethanol at pH ~ 2.5 and 65°C. Under these conditions the proteins are completely unfolded, the disulfide bonds are reduced and they give sharp PMR spectra (*20*). This spectrum was subtracted from that of a sample of the protein which had not been subjected to the exchange treatment.

The results in Table 3 show that there was no detectable exchange of phenylalanyl or tyrosyl protons and a small degree of exchange of the C-2 histidine protons and some tryptophyl protons at the highest temperature used, 70°C. The exchange of the C-2 histidine proton does not normally occur at pH < 5 (*9*), but at higher temperatures some exchange can occur as a result of a second mechanism of reaction, which involves direct D_2O attack on the imidazolium ring (*21*). The limited exchange of tryptophyl protons is probably due to acid catalysis (*13*) rather than platinum catalysis, since it has been found (*15*) that the exchange of tryptophan in peptides is slower than that of phenylalanine and tyrosine and neither of the latter exchanged under the conditions of the experiments.

The absence of exchange of aromatic protons of tyrosine [observed by Murray (*15*) with the amino acid] and phenylalanine in proteins is disappointing. In a native protein such as lysozyme it could be attributed to the fact that aromatic amino acids tend to be buried in the interior of the protein and those few that are located on the surface (*22*) are not sufficiently exposed to permit interaction of the aromatic ring with the surface of the catalyst. It was hoped that exchange might occur in unfolded proteins, the aromatic side chains of

TABLE 3
ATTEMPTED EXCHANGE OF PROTEINS IN D_2O USING PLATINUM CATALYSTS

Substrate[a]	Solvent	Catalyst	Conditions of treatment		Extent of exchange of aromatic protons
			Temp (°C)	Time (days)	
Lysozyme[b]	D_2O	A	37	4.25	Nil
Lysozyme[c]	D_2O, pH 3.9	B	37	5	Nil
Lysozyme[c]	D_2O, pH 1.3	B	50	2	Nil
Lysozyme[c]	D_2O, pH 2.8	B	70	1	Partial exchange of C-2 His proton and of tryptophyl protons
Lysozyme[d]	D_2O, pH 2.8	B	70	2	
Lysozyme[c]	6.5 *M* GuDCl, pH 2.8	B	50	2	Nil
Ribonuclease[e]	D_2O, pH 2.7	B	70	2	20% exchange of His C-2, but no exchange of His C-4, Phe or Tyr protons

[a] Hen egg white lysozyme (twice recrystallized) and bovine pancreatic ribonuclease-A (phosphate free) were obtained from Worthington Biochemicals and were lyophilized twice from D_2O before use to remove readily exchangeable protons.

[b] Lysozyme (70 mg) in 1.4 ml D_2O was treated with 5 mg of catalyst A.

[c] Lysozyme (100 mg) in 2 ml D_2O was treated with 10–15 mg of catalyst B.

[d] Lysozyme (100 mg) in 2 ml D_2O was treated with 80 mg of catalyst B.

[e] Ribonuclease (56 mg) in 1 ml D_2O was treated with 21 mg of catalyst B.

which are more exposed since these proteins exist in solution essentially as random coils with disulfide cross links intact. Initially GuDCl was used as the denaturant (*18*), but this reagent inhibits the catalytic exchange reaction, at least in phenylalanine (Tables 1 and 2). To overcome this problem the proteins were unfolded by heating. At pH 2.8 and 70°C, lysozyme and ribonuclease are both extensively unfolded (*20,23*), yet the phenylalanyl and tyrosyl side chains of neither protein showed any evidence of metal-catalyzed exchange.

Lack of platinum-catalyzed deuterium exchange in the denatured protein is probably caused by one or more of the following factors:

1. Poisoning of the catalyst by irreversible adsorption of the protein on its surface. This could be investigated further by variation of the protein/catalyst ratio and sorption studies on the catalyst.

2. Steric hindrance by adjacent side chains in the protein which could prevent adsorption of the aromatic side chains onto the surface of the catalyst. Murray (*15*) found that steric hindrance was important in determining the exchange rates in small peptides.

3. The slow translational motion of the protein chain in solution compared with a small peptide, which allows fewer collisions per unit time between the aromatic rings and the catalyst and thereby decreases the rate of reaction.

Clearly, further studies are needed to decide the relative importance of these and possibly other factors. However, it is evident that catalysis of isotopic exchange by reduced platinum oxide is not useful for proteins, despite its success with free amino acids and small peptides.

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REFERENCES

1. Roberts, G. C. K., and Jardetzky, O., *Advan. Protein Chem.* **24,** 447 (1970).
2. King, N. L. R., and Bradbury, J. H., *Nature (London)* **229,** 404 (1971).
3. Seiter, C. H. A., Feigenson, G. W., Chan, S. I., and Hsu, M-C., *J. Amer. Chem. Soc.* **94,** 2535 (1972).
4. Campbell, I. D., Dobson, C. M., Williams, R. J. P., and Xavier, A. V., *J. Magn. Res.* **11,** 172 (1973).
5. Markley, J. L., Putter, I., and Jardetzky, O., *Science* **161,** 1249 (1968).
6. Crespi, H. L., and Katz, J. J., *Nature (London)* **224,** 560 (1969).
7. Bradbury, J. H., and King, N. L. R., *Aust. J. Chem.* **25,** 209 (1972).
8. Bradbury, J. H., and Chapman, B. E., *Biochem. Biophys. Res. Commun.* **49,** 891 (1972).
9. Bradbury, J. H., Chapman, B. E., and Pellegrino, F. A., *J. Amer. Chem. Soc.* **95,** 6139 (1973).
10. Bradbury, J. H., and Norton, R. S., *Eur. J. Biochem.*, in press.
11. Bak, B., Dambmann, C., and Nicolaisen, F., *Acta. Chem. Scand.* **21,** 1674, (1967).
12. Bak, B., Led, J. J., and Pedersen, E. J., *Acta Chem. Scand.* **23,** 3051 (1969).
13. Norton, R. S., PhD thesis, Australian National Univ., 1974.
14. Calf, G. E., Garnett, J. L., Halpern, B. H., and Turnbull, K., *Nature (London)* **209,** 502 (1966).
15. Murray, S., MSc thesis, Wollongong Univ. College, 1973.
16. Calf, G. E., and Garnett, J. L., *J. Phys. Chem.* **68,** 3887 (1964).
17. Garnett, J. L., and Sollich, W. A., *J. Catal.* **2,** 339 (1963).
18. Tanford, C., *Advan. Protein Chem.* **23,** 121 (1968).
19. Bradbury, J. H., and King, N. L. R., *Aust. J. Chem.* **22,** 1083 (1969).
20. Bradbury, J. H., and Norton, R. S., *Int. J. Protein Peptide Res.* **6,** 295, (1974).
21. Vaughan, J. D., Mughrabi, Z., and Wu, E. C., *J. Org. Chem.* **35,** 1141 (1970).
22. Imoto, J., Johnson, L. N., North, A. C. T., Phillips, D. C., and Rupley, J. A., *in* "The Enzymes" (P. D. Boyer, Ed.), 3rd ed., Vol. 7, p. 665. Academic Press, New York, 1972.
23. Richards, F. M., and Wyckoff, H. W., *in* "The Enzymes" (P. D. Boyer, Ed.), 3rd ed., Vol. 4, p. 647. Academic Press, New York, 1971.