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Hydrogen–deuterium exchange of small peptides in aqueous solution

Measurements of the rate of hydrogen–deuterium exchange in non-intramolecularly hydrogen-bonded peptides and peptide analogs in aqueous solution will greatly facilitate the quantitative interpretation[1,2] of H–D exchange in proteins and polypeptides as measured with the exchange technique of LINDERSTRØM-LANG[3]. A recent communication[4] has reported some preliminary infrared measurements of the H–D exchange in N-methylacetamide. Such studies have now been extended to some simple peptides.

To measure the rate of the exchange reaction of the peptide group

$$-CO-NH- + D_2O \rightarrow -CO-ND- + HDO$$

125 μl of a 2 % solution of the peptide in H_2O was lyophilized in a small test tube over conc. H_2SO_4. Subsequently a 1-ml syringe with a spring-loaded piston was filled with approximately 230 μl 99.8 % D_2O containing either small amounts of buffer acids and salts or of HCl. The lyophilized peptide was quickly dissolved by emptying the syringe into the test tube via a special two-way valve with a 0.3-mm bore and pumping the solution up and down a few times. Immediately following this operation the valve was turned, allowing the reaction mixture to be forced directly into a 0.10-mm optical cell with fluorite windows placed in the beam of a Perkin-Elmer Model 13U Infrared Spectrometer arranged for single-beam operation with a fluorite prism. In order to keep the cell temperature constant, thermospacers at 22° were used. The exchange reaction was followed by recording the increasing transmission at 1580 cm^{-1} (essentially the disappearance of the amide II band of the protonated peptide group). In the majority of cases it was possible to start following the course of the exchange reaction approximately 10 sec after its inception. "pD" was determined in the combined reaction mixtures from duplicate runs as described elsewhere[5].

With dipeptides (Gly–Gly, Gly–Ala, Gly–Leu, Ala-Gly, Ala–Ala) the exchange reaction of the peptide group was found to follow first-order kinetics up to at least 75 % conversion. (Amine and carboxyl hydrogen atoms exchange at a rate too fast

to be measured by this technique.) With tripeptides (Gly–Gly–Gly, Ala–Gly–Gly, Leu–Gly–Gly) the course of the exchange reaction as recorded did not in general correspond to simple first-order kinetics, but the exchange data could be accounted for within experimental error when it was assumed that the exchange reactions of the two peptide groups in the tripeptide molecule proceeded according to different first-order rate laws. At some pD's it was possible to assign definite values to the two rate constants involved. Table I summarizes some of the results obtained.

The variation of the rate constants in Table I with pD resembles that obtained previously with N-methylacetamide[1] in that there is a minimum in rate and that on the acid side of the rate minimum first-order acid catalysis is observed. However, instead of the minimal rate occurring around pD 5.4 in the case of N-methylacetamide it occurs in Gly–Gly at pD about 2.4 and in Ala–Gly–Gly at pD about 2.2 (N-terminal peptide group) and pD about 3.0 (C-terminal peptide group). A detailed discussion of the assignment of the measured rate constants to the two peptide groups in Ala–Gly–Gly in Table I will appear in *Compt. rend. trav. Lab. Carlsberg*. The exchange

TABLE I

FIRST-ORDER RATE CONSTANTS OF THE H–D EXCHANGE REACTIONS IN GLY–GLY AND ALA-GLY–GLY

Gly Gly		*Ala-Gly Gly*		
			N-terminal –CO–NH–	*C-terminal* –CO–NH–
pD	min^{-1}	*pD*	min^{-1}	min^{-1}
1.10	4.1	1.14	1.4	
1.33	2.0	1.22	0.97	
1.95	0.58	1.34	0.86	
2.01	0.60	1.67	0.45	5.6
3.14	1.06	2.03	0.30	2.6
3.84	3.6	3.38		0.71
		4.14		0.91

behaviour of the di- and tripeptides investigated relative to that of N-methylacetamide is in apparent agreement with the exchange mechanism proposed by BERGER and coworkers[6], when the effects of electron-withdrawing substituents on the amide group is taken into account.

Our results do not appear to be compatible with some measurements by the Linderstrøm-Lang exchange technique of the rate of H–D exchange in deuterated triglycine at 0° and pH 3 in water. It was reported[3] that 80 % of the peptide hydrogen atoms had exchanged within the first 0.8 min following dissolution of the peptide in water. In contrast, the data in Table I and additional data on triglycine suggest that this H–D exchange in triglycine should be considerably slower. This discrepancy between the two methods will be briefly discussed in the following note.

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Hydrogen–deuterium exchange of poly-DL-alanine in aqueous solution

It has been concluded from infrared spectra of films of PDLA that it exists in aqueous solution in the α form[1]. Assuming an α-helical model, BERGER AND LINDERSTRØM-LANG were able to account for most of their H–D exchange results obtained with PDLA in aqueous solution[2,3]. As the H–D exchange mechanism of a polypeptide helix would be of considerable interest, the present study was undertaken in an attempt to clarify the exchange mechanism of PDLA in aqueous solution.

The experimental method was essentially that described in the preceding note[1]. The same PDLA preparation used by BERGER AND LINDERSTRØM-LANG[2] (containing approximately 30 residues per molecule, DP = 30) was dissolved in dilute NaOH and lyophilized over conc. H_2SO_4 to remove a pyridine contaminant. 99.8% D_2O containing varying small amounts of HCl, sodium citrate and citric acid was added to give a solution containing 1% PDLA, and the exchange reaction (A) was followed at 1550 cm^{-1}. Using an analogous procedure, the course of the exchange reaction (B) between deuterated PDLA and H_2O was followed by recording the decreasing infrared transmission of the reaction mixture at 2570 cm^{-1} against time[5]. Thermospacers maintained the cell temperature at 10° or 22°.

The recorded infrared transmission T_t approached asymptotically a constant value $T\infty$. When plotting loglog $(T\infty/T_t)$ (1550 cm^{-1}) or loglog $(T_t/T\infty)$ (2570 cm^{-1}) against time our results in typical cases gave linear plots up to at least 75% of total conversion. From the slopes of these plots apparent first-order rate constants were calculated, and some of these are presented in Table I.

Our results do not appear to be compatible with those of BERGER AND LINDERSTRØM-LANG[2] obtained with PDLA (DP = 30) at 0°, 10° and 20°. None of their exchange curves, giving degree of H–D exchange as a function of time, could be fitted to one exponential decay function as is the case with the data summarized in Table I. It has already been reported[1] that the exchange technique of LINDERSTRØM-LANG[6] does not give good agreement with the present infrared technique in measurements of the rate of H–D exchange in triglycine. This situation suggested to us that the exchange technique of LINDERSTRØM-LANG might indicate too high initial rates of H–D exchange. Preliminary control experiments have supported this suggestion in demonstrating that in some instances H–D exchange occurs during the sublimation involved. This phenomenon may account for the deviation of the exchange curves of BERGER AND LINDERSTRØM-LANG[2] from simple first-order curves. In this connection it should be pointed out that at a given pH and temperature their experimental points, neglecting zero-time values, fall within experimental

Abbreviation: PDLA, poly-DL-alanine.