[1] W. P. Bryan and S. O. Nielsen, *Compt. rend. trav. Lab. Carlsberg*, in preparation.
[2] W. P. Bryan and S. O. Nielsen, *Biochim. Biophys. Acta*, 42 (1960) 552.
[3] K. Linderstrøm-Lang, in A. Neuberger, *Symposium on Protein Structure*, Methuen, London 1958, p. 23.
[4] S. O. Nielsen, *Biochim. Biophys. Acta*, 37 (1960) 146.
[5] K. Mikkelsen and S. O. Nielsen, *J. Phys. Chem.*, 64 (1960) 632.
[6] A. Berger, A. Loewenstein and S. Meiboom, *J. Am. Chem. Soc.*, 81 (1959) 62.

Received June 27th, 1960

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.

error on a simple first-order exchange curve. Agreement exists between the rate constants calculated from these curves and the rate constants presented in Table I. A detailed discussion of this and the following points will appear in *Compt. rend. trav. Lab. Carlsberg.*

TABLE I

FIRST-ORDER RATE CONSTANTS OF THE H-D EXCHANGE OF PDLA

PDLA (DP 30)			
22°		*10°*	
pD	k_A *(min*$^{-1}$*)*	*pD*	k_A *(min*$^{-1}$*)*
1.64	1.27	2.07	0.23
2.37	0.37	4.67	0.21
2.56	0.26		
3.69	0.144		
4.62	0.58		

PDLA (DP 20), 22°			
pD	k_A *(min*$^{-1}$*)*	*pH*	k_B *(min*$^{-1}$*)*
2.24	0.48	1.93	0.50
4.63	0.68	4.12	0.65

In view of the present results as well as recent infrared spectral work[7] it would appear that the question whether PDLA exists in aqueous solution as an α-helix would have to be reconsidered. The following observations made during the course of the present investigation weigh against a substantial fraction of PDLA being in the α-helical form in aqueous solution between 0° and 22°. (1) The H-D exchange of PDLA behaves like a simple first-order reaction. (2) The exchange rate is of the same order of magnitude as that found in simple di- and tripeptides[4]. (3) Additions of urea[2] and dioxane have only a small effect on the exchange rate. (4) The rate of H-D exchange in poly-α-L-glutamic acid in solution is very slow under conditions[8] where helix formation occurs.

We are greatly indebted to the late Professor K. LINDERSTRØM-LANG, under whose inspiring leadership this investigation was initiated. A gift of poly-α-L-glutamic acid from Professor R. LUMRY is gratefully acknowledged.

Carlsberg Laboratory, Chemical Department,
Copenhagen (Denmark)

WILLIAM P. BRYAN
SIGURD O. NIELSEN*

[1] A. ELLIOTT, *Nature*, 170 (1952) 1066.
[2] A. BERGER AND K. LINDERSTRØM-LANG, *Arch. Biochem. Biophys.*, 69 (1957) 106.
[3] K. LINDERSTRØM-LANG, *Acta Chem. Scand.*, 12 (1958) 851.
[4] S. O. NIELSEN, W. P. BRYAN AND K. MIKKELSEN, *Biochim. Biophys. Acta*, 42 (1960) 550.
[5] S. O. NIELSEN, *Biochim. Biophys. Acta*, 37 (1960) 146.
[6] I. M. KRAUSE AND K. LINDERSTRØM-LANG, *Compt. rend. trav. Lab. Carlsberg, Ser. chim.*, 29 (1955) 367.
[7] A. ELLIOTT, *Advances in Spectroscopy*, 1 (1959) 214.
[8] P. DOTY, A. WADA, J. T. YANG AND E. R. BLOUT, *J. Polymer Sci.*, 23 (1957) 851.

Received June 27th, 1960

---

* Present address: Danish Atomic Energy Commission, Research Establishment, Chemistry Dept., Risø (Denmark).