EXCHANGE OF HYDROGEN ATOMS IN INSULIN WITH DEUTERIUM ATOMS IN AQUEOUS SOLUTIONS

by

AASE HVIDT AND K. LINDERSTRØM-LANG

Carlsberg Laboratorium, Copenhagen (Denmark)

In an attempt to throw light upon the physical state of insulin in aqueous solutions a determination was made of the number of hydrogen atoms in this hormone which can be exchanged for deuterium atoms from a medium of 100% deuterium oxide. Carefully purified and dried pork insulin (from Nordisk Insulin Laboratory) was allowed to react at 37° C with 99.6% deuterium oxide at pH 3. The protein concentration was 2%. At suitable times the exchange process was stopped in samples of the solution by freezing them to —80° C. The excess water was removed by lyophilisation and drying in vacuum over P_2O_5 for several days. The deuterium content of the dry protein was determined by letting it react again with ordinary water and estimating the resulting deuterium-content of this water by equilibration of the solution with pure water through an air gap. The density of the pure water was determined in the gradient tube. Details of the method have been described[1].

The results are seen in Fig. 1, where the number of replaced hydrogen atoms per mole of insulin (M.W. = 5777) is plotted against time. According to SANGER, THOMPSON AND TUPPY[2] and HARFENIST[3] pork insulin on the basis of a minimum molecular weight of 5777 may exchange the number of hydrogen atoms given in Table I. A comparison with Fig. 1 demonstrates the fact that out of the possible 92 hydrogen atoms only 46 exchange even after prolonged reaction (124 hours). It is reasonable to assume that it is the amide hydrogens of the 48 peptide bonds which are protected, and since the peptide bond hydrogen in shorter peptides like leucyltriglycine exchange readily though at a finite rate under similar conditions, our results may be taken to confirm the existence of stable internal hydrogen bonds between the CO- and NH-groups of the backbone in dissolved insulin. If the monomer of insulin is considered to be a double α-helix consisting of one A-chain and one B-chain, there are 6 terminal peptide NH-groups which are unlinked and should exchange. These groups—or some of the groups of the side chains—may, however, be masked in the associated molecules existing at pH 3, so that our results are compatible with this model.

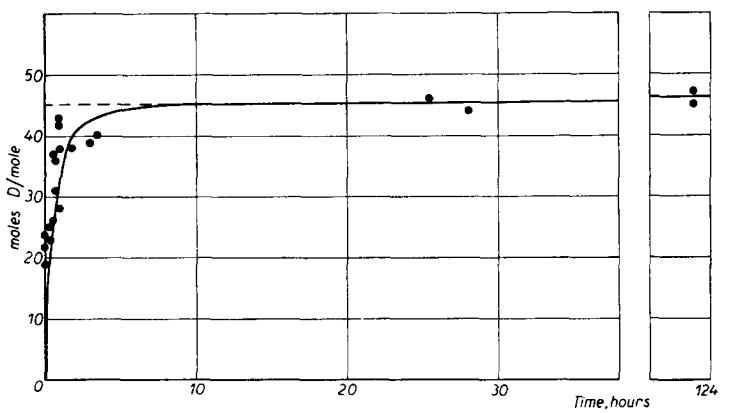

Fig. 1.

Preliminary investigations of the sodium salt of the isolated oxidized A-chain have given the results shown in Table II. It will be seen that all the hydrogen atoms of the peptide bonds exchange in this substance, a fact which emphasizes the importance of the tertiary structure[4] of the native insulin molecules for the stability of the internal hydrogen bonds.

TABLE I

THEORETICAL NUMBER OF EXCHANGEABLE HYDROGEN ATOMS IN INSULIN

Groups with exchangeable hydrogen atoms	*Number of groups*	*Number of easily exchangeable hydrogen atoms*	*Number of more firmly bound hydrogen atoms*
1. Amino acid side chains			
asparagine	3	—	6
threonine	2	—	2
serine	3	—	3
glutamine	3	—	6
glut. acid	4	4	—
tyrosine	4	4	—
histidine	2	2	—
lysine	1	2	—
arginine	1	5	—
2. Endgroups			
glycine	1	2	—
phenylalanine	1	2	—
asparagine	1	1	—
alanine	1	1	—
3. Backbone			
—CO—NH—	48	—	48
4. HCl used to bring pH to 3	4	4	—
	Total	27	65

TABLE II

EXCHANGE OF HYDROGEN ATOMS IN A-CHAIN-Na_4 (M.W. = 2661)

Theoretical number of exchangeable hydrogen atoms:

Side-chains and endgroups	18
Peptide hydrogen	20
Total	38

Experimental results:

Time hours	*moles D/mole A-chain*
0.1	32
0.5	34
1.5	35
2	35
18	34
22.5	34
23	35
24	38
24.5	38

REFERENCES

1 AASE HVIDT, G. JOHANSEN, K. LINDERSTRØM-LANG AND FRED VASLOW, *Compt. Rend. Lab. Carlsberg, Sér. chim.*, 29 (1954) 9.

2 F. SANGER, E. O. P. THOMPSON AND H. TUPPY, *Symposium sur les Hormones Protéiques et Dérivées des Protéines*, (1952) 26.

3 E. J. HARFENIST, *J. Am. Chem. Soc.*, 75 (1953) 5528.

4 K. LINDERSTRØM-LANG, *Lane Medical Lectures*, Stanford University Press, (1952) p. 53.

Received June 3rd, 1954