Deuterium exchange and reversible denaturation of α-chymotrypsinogen

Measurements of deuterium exchange in aqueous solution have demonstrated that native β-lactoglobulin[1] and whale myoglobin[2] each contain a large number of hydrogen bonds which show no exchange of hydrogen atoms with water. Bovine α-chymotrypsinogen likewise may be shown to contain hydrogen bonds of extraordinary stability. Heat denaturation at low pH will result, however, in complete exchange of the hydrogen of these bonds. EISENBERG AND SCHWERT[3] found that this protein exists at pH 2 in two forms in equilibrium, the native and the reversibly denatured, distinguished by the insolubility of the latter in 1 *M* NaCl at pH 3. The purpose of the experiments reported here was to explore the relationship, if any, between the stability of hydrogen bonds in α-chymotrypsinogen and the transformation between the native and reversibly denatured states.

Calculations based on determinations of composition[4], molecular weight[5], and terminal residues[6] of α-chymotrypsinogen give 404 as the total number of theoretically exchangeable hydrogen atoms per mole of isoionic protein. Peptide linkages carry 232 of these atoms. At pH 3.4 about 11 additional hydrogens are bound.

The experimental techniques by which deuterium exchange was measured have been described in detail elsewhere[7,8]. At pH 3.4 and 39°, α-chymotrypsinogen in D_2O did not exchange all of the theoretically exchangeable hydrogen even during a period of 95 h. However, when deuteration was carried out at 50°, the number of exchanged deuterium atoms reached 397 with an average deviation of 5 in eight experiments.

Complete deuteration occurred rapidly at 39° near pH 2, where reversible denaturation is appreciable. Control tests confirmed the statement by SCHWERT[9] that the transition to reversibly denatured protein occurs under similar conditions in H_2O and in D_2O. In order to assess the role of irreversible denaturation, deuterium-exchange measurements were coupled with measurements of solubility in 1 *M* NaCl. Table I presents typical experiments in which deuteration was carried out in 99.78 %

TABLE I

DEUTERIUM EXCHANGE AND DENATURATION OF α-CHYMOTRYPSINOGEN

Expt.	1 Deuteration, 39°		2	3 Back-exchange, pH 3.4		4	5
	pH	time (min)	Irreversible denaturation %	temp.	time (h)	n atoms D/mole	Δ n
1	2.0	30	58	50	20	399, 397	20
2	2.0	30		39	20	377, 379	
3	2.0	15	34	50	20	402, 399	22
4	2.0	15		39	20	380, 378	
5	2.0	5	21	50	20	369, 372	19
6	2.0	5		39	20	350, 354	
7	2.2	15	14	50	42	392, 388	26
8	2.2	15		39	42	363, 365	
9	2.2	9	12	50	42	370, 375	21
10	2.2	9		39	42	350, 354	
11	2.5	1440	28	50	20	377, 380	32
12	2.5	1440		39	20	348, 345	

D_2O under conditions given in column 1. Samples of the deuterated protein were stored at 5° overnight. The percentage of protein which then precipitated in 1 *M* NaCl at pH 3 is given in column 2. Back-exchange was conducted in ordinary water buffered near pH 3.4 with 0.015 *M* glycine. In column 4 the number, *n*, of deuterium atoms found in the cryosublimate per mole of protein is given for duplicate experiments. Back-exchange at 50° released the total deuterium in the deuterated protein, while back-exchange at 39° gave values of *n* which represent total deuterium less that amount bound in the most stable form. The last column lists the difference between average values for back-exchange at 50° and 39°.

The sharp transition in deuteration as the pH is varied from 2.0 to 2.5 is noteworthy. A sharp temperature transition between 39° and 50° is evident in the data for back-exchange. These transitions in deuterium exchange occur in the same regions of pH and temperature as the transitions in equilibrium between native and reversibly denatured protein[3]. Irreversible denaturation is not necessary for complete exchange. When conditions are readjusted after deuteration so as to shift the equilibrium far to the side of native protein (pH 3.4 and 39°), it is possible to trap a large number of D atoms in protected positions (column 5).

Apparently the stability of at least 40 hydrogen bonds in α-chymotrypsinogen is drastically altered in a reversible fashion when the protein passes from the native to the reversibly denatured state. It may be recalled that SCHELLMAN[10] could detect no change in optical rotation or dispersion over the range of pH and temperature in which the transition occurs. STAUFF AND RASPER[11], however, have recently discovered small but definite changes in a number of other physical properties.

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1 K. LINDERSTROM-LANG, *Soc. Biol. Chemists, India, Silver Jubilee Souvenir*, (1955) 191.
2 E. BENSON AND K. LINDERSTROM-LANG, *Biochim. Biophys. Acta*, 32 (1959) 579.
3 M. A. EISENBERG AND G. W. SCHWERT, *J. Gen. Physiol.*, 34 (1951) 583.
4 P. E. WILCOX, E. COHEN, W. TAN, *J. Biol. Chem.*, 228 (1957) 999.
5 P. E. WILCOX, J. KRAUT, R. D. WADE AND H. NEURATH, *Biochim. Biophys. Acta*, 24 (1957) 72.
6 J. F. PECHERE, G. H. DIXON, R. H. MAYBURY AND H. NEURATH, *J. Biol. Chem.*, 233 (1958) 1364.
7 A. HVIDT, G. JOHANSEN, K. LINDERSTROM-LANG AND F. VASLOW, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 29 (1954) 129.
8 I. M. KRAUSE AND K. LINDERSTROM-LANG, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 29 (1955) 367.
9 G. W. SCHWERT, *J. Cell. Comp. Physiol.*, Suppl. 1, 47 (1956) 126.
10 J. A. SCHELLMAN, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 30 (1958) 450.
11 J. STAUFF AND J. RASPER, *Kolloid Z.*, 159-2 (1958) 97.

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