GRADUAL EXPOSURE OF CYSTINE SIDE-CHAIN RESIDUES DURING UREA DENATURATION OF HUMAN SERUM ALBUMIN

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Showing the accessibility of disulfide groups in the protein molecule, the polarographic catalytic hydrogen current (Brdička current) was employed for the investigation of the urea denaturation of human serum albumin. The stepwise character of the denaturation curve was accounted for the gradual conformational changes of the protein molecule and related increasing accessibility of cystine side-chain residues.

In spite of a number of papers on the urea denaturation of human and bovine serum albumin (HSA, BSA) the nature of this reaction is still far to be understood completely. The conclusions drawn from the results of different methods are often in disagreement. The denaturation is observed to be either a very rapid (immediate) process (optical rotation[1] and UV-spectrophotometry[2]) or rapid reaction at first followed by a slower one (viscosimetry[1]) or a gradual reaction (electrophoresis[3,4]).

The denaturation of proteins by various agents was also studied polarographically[5–7]. In that case the Brdička current (review see[8,9]) occurring in the presence of some proteins and Co(II) or Co(III) ions is considered to be proportional to the accessibility of the cystine side-chain residue in the protein molecule.

In the present paper we have studied the kinetics of the urea denaturation of HSA using the polarographic method.

EXPERIMENTAL

Chemicals

Human serum albumin, a product from Imuna (Šarišské Michalany, Czechoslovakia) was of high electrophoretic purity. Urea of analytical grade purity (Lachema, Brno) was recrystallised from aqueous ethanol. Owing to its hydrolysis the stock solution (9M, pH 7·2) was always freshly prepared. Other chemicals (Lachema, Brno) were of analytical grade purity.

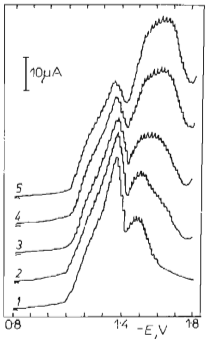

FIG. 1

Polarographic Curves of HSA Denaturated by 8M Urea

1 Native protein; time of denaturation: *2* 5; *3* 20; *4* 90; *5* 200 min. Concentration of HSA $3 \cdot 5 \cdot 10^{-7}$ mol/l in 0·1M-NH_4Cl; 1M-NH_3; 1 mM-$Co(NH_3)_6 \cdot Cl_3$.

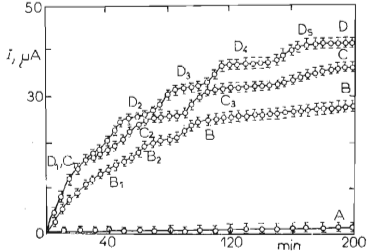

FIG. 2

Dependence of Brdička Current of HSA ($3 \cdot 5 \cdot 10^{-7}$ mol/l) on Denaturation Time

Urea concentration A 2; B 4; C 6; D 8 mol/l.

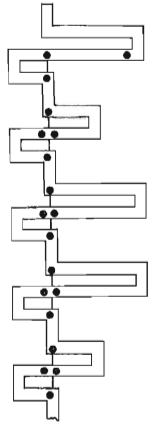

FIG. 3

Location of Half-Cystine Residues in HSA Molecule According to ref.[15]

Polarography

Polarographic measurements were carried out using Polariter PO—4 polarograph (Radiometer, Copenhagen) in the Kalousek-type cell with saturated calomel electrode (S.C.E.) to which all potential values are referred. The capillary of dropping mercury electrode had following constants (at the mercury column height of 70 cm): the flow rate of mercury $m = 3{\cdot}75$ mg s^{-1} and the drop time $t_1 = 2{\cdot}03$ s at —1·55 V in the supporting electrolyte containing 0·1M-NH_4Cl, 1M-NH_3 and 1 mM-$Co(NH_3)_6Cl_3$ (pH 10·3).

Denaturation

The required quantity of protein solution (in water) was added to the urea solution so that the resulting concentration of HSA in the mixture was $7 \cdot 10^{-5}$ mol/l and that of urea 2, 4, 6 and 8 mol/l, respectively. After an intensive mixing the samples (50 µl) were taken from the denaturation mixture in fixed time intervals (5 minutes) and added to the supporting electrolyte (10 ml). The resulting concentration of HSA in the polarographic cell was $3{\cdot}5 \cdot 10^{-7}$ mol/l.

All experiments were carried out at 22°C.

RESULTS AND DISCUSSION

The polarograms of HSA denaturated by 8M urea are presented in Fig. 1. Following the reduction wave of cobalt, the Brdička current is situated at about −1·6 V. Its height at −1·63 V (in µA) referred to that of the native form (curve *1*) was taken to quantify the denaturation process. The time dependence of denaturation of HSA by 2,4,6 and 8M urea, respectively, is shown in Fig. 2 (with the standard deviations of 10 measurements). The curves have a stepwise character due to a gradual increase of accessibility of —S—S— groups in the molecule of HSA.

The molecule of HSA contains one —SH and 17 —S—S— groups. *i.e.* 35 catalytically active —SH groups (an accessible —S—S— group is reduced at the electrode to two —SH groups at potentials preceding the potential range of the Brdička current[10,11]). These groups are located in the molecule as follows from the Fig. 3.

The stepwise character of the denaturation curves (Fig. 2) is considered to be due to gradual unfolding of molecule of HSA under the influence of urea and to the exposure of buried disulfide groups. At first sight it is seen that the exposure of —S—S— groups comes "quantum by quantum". This quantum is believed to be related to one tetrad of half-cystine residues. As we know the Brdička current corresponding to one half-cystine residue[12] (1·24 µA per —SH for our electrode), we are able to calculate the number of half-cystine residues liberated in every denaturation step from the Fig. 2. In the initial stage of denaturation the simultaneous exposure of several tetrads of half-cystine residues probably occurs (step B_1, C_1, D_1 ~3; C_2, D_2 ~2 tetrads). In the latter stage the separate tetrads are gradually made free in steps B_2, B_3, C_3, D_3, D_4, D_5 ~1. The exposure of half-cystine residues by tetrads is in agreement with the location of half-cystine residues in the primary structure of HSA (Fig. 3)[13–15].

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