COLOUR REACTIONS OF NATIVE AND DENATURED PROTEINS

by

FELIX HAUROWITZ AND SEVKET TEKMAN

Department of Medical and Biological Chemistry, University of Istanbul (Turkey)

Native and denatured proteins differ from each other by their physical-chemical properties, chiefly by their solubility. One of the few chemical reactions, by which native and denatured proteins can be differentiated from each other, is the nitro-prusside test. While denatured ovalbumin gives an intensive pink colour with nitroprusside, no colour is observed in solutions of native ovalbumin[1, 2, 3]. Two different explanations are possible for the positive nitroprusside test of denatured proteins:

I. The SH-group found in the denatured protein are presented also in the native proteins molecule, but inaccesible to the reagent because of the tightly folded structure of the peptide chains; they become accessible to the reagent by unfolding of the peptide chains during the denaturation.

II. The SH-groups found in the denatured protein are formed during the denaturation by chemical changes, as for instance cleavage of dithio-, of thioester- or of other S-containing groups.

If the first assumption is right the constitution of the protein molecule in the native and the denatured state is the same and they differ from each other only by the more or less tight folding of their peptide chains; one should expect in this case that the intensity of other colour reactions also would increase during the denaturation. This was examined in the present paper.

EXPERIMENTS

The following proteins were examined: a) Ovalbumin from hens eggs[4], b) beef serum albumin, c) beef serum globulin, both of them prepared by fractional precipitation with ammonium sulfate, d) lactoglobulin from cows milk[5]. Denaturation was achieved by mixing 2 ml (24 mg) of the aqueous protein solution with 3 ml of a buffer solution p_H 9.0[6], and keeping the mixture in a boiling water bath for 10 or for 30 minutes.

The biuret reaction was carried out according to HARRISON[7], the SAKAGUCHI test for arginine according to[8] and the VOISINET test for tryptophane according to FÜRTH[9]. No differences were observed between the colours given by native and by denatured proteins.

TABLE I

INTENSITY OF THE DIAZO REACTION

Protein	Extinction coefficients ε_8 mm		
	Native	10 minutes denatured	30 minutes denatured
Ovalbumin	0.14	0.40	0.43
Serum Albumin	0.27	0.34	0.35
Serum Globulin	0.33	0.37	0.41
Lactoglobulin	0.41	0.47	0.48

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Diazo reaction

10 ml (24 mg) of the protein solutions were mixed with 3.6 ml of the soda solution and 1.5 ml of the diazo-solution of KOESSLER AND HANKE[10]. The red colour was measured with the PULFRICH step photometer, using the colour filter S_{53} (s. Table I).

1.5 ml of a 8.4 % solution of ovalbumin were mixed with 4.5 ml of a 60 % solution of urea and kept at room temperature for 24 hours. The urea was removed by dialysis. 5 ml of a solution containing 12 mg of the denatured protein were mixed with 1.8 ml of the soda solution and 0.75 ml of the diazo-solution[10] and measured as described above. The extinction coefficient of the denatured ovalbumin was ε_6 mm = 0.35, that of a control solution containing native ovalbumin 0.22.

Phenol reaction[11]

Solutions containing 10 mg of the protein in 25 ml of 0.008-N NaOH were used. Two ml of these solutions were mixed with 6 ml of a saturated solution of sodium carbonate and 1 ml of 0.1 % $CuSO_4$. The test tubes (a) were cooled immediately by an ice bath, while the second test tube (b) was kept for 5 minutes in a boiling water bath, than cooled down. To both of the solutions (a) and (b) 1 ml of the diluted phenol reagent (1 : 3) was added[11]. The blue colour was measured after 30 minutes in the step photometer using the colour filter S_{72} and 10 mm vessels (Table II).

TABLE II

COLOUR REACTION ACCORDING TO FOLIN-WU-CIOCALTEU[11]

Protein	Extinction coefficients	
	Native protein	Denatured proteins
Ovalbumin	0.50	0.81
Serumalbumin . .	0.45	0.62
Serumglobulin . .	0.53	0.81
Lactoglobulin . .	0.46	0.64

DISCUSSION

Proteins denatured by keeping at 100° at a p_H of 9.0 give a more intensive colour with diazo-benzene-sulfonic acid than native proteins. This cannot be attributed to a partial hydrolysis of the protein. For, the same increase of the colour intensity is observed if the denaturation is brought about by urea at room temperature.

Denatured proteins give also a more intensive colour reaction with FOLIN's phenol reagent; similar observations on trypsinogen and ovalbumin have been made previously by HERRIOT[12].

If the biuret reaction, the arginine reaction (SAKAGUCHI), and the tryptophane reaction (VOISINET) of native and of denatured proteins are compared, no marked difference is found. This is due to the fact that the native proteins are denatured by the alcaline contained in the first two of the mentioned reagents or by the strong HCl of the Voisinet reagent.

Our experiments demonstrated that the increased intensity of the nitroprusside test observed after the denaturation of ovalbumin is accompanied by an increased intensity of the diazo and the phenol reaction. This is best explained by the assumption that not only SH groups but also phenolic OH groups and, probably, other atomic groups of the native protein molecule are inaccessible to the chemical reagents because of sterical hindrance exerted by the tightly folded peptide chains and that these groups become accessible after denaturation by unfolding of the peptide chains[13, 14].

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SUMMARY

The colour reaction given by denatured ovalbumin, serum albumin, serum globulin and lactoglobulin with diazobenzene sulfonic acid or with FOLIN's phenol reagent is more intensive than the colour reaction given by the same proteins in the native state. The weak reaction given by native proteins is attributed to steric hindrance due to the tight folding of the peptide chains in the native protein molecules.

RÉSUMÉ

La réaction colorée donnée par l'ovalbumine, la sérumalbumine, la sérumglobuline avec l'acide diazobenzène-sulfonique ou avec le réactif des phénols de FOLIN, est plus intense lorsqu'il s'agit des protéines dénaturées que lorsqu'il s'agit des protéines natives. La faiblesse de la réaction donnée par les protéines à l'état natif est attribuée à l'empêchement stérique dû au repliement considérable des chaînes peptidiques dans les molécules des protéines natives.

ZUSAMMENFASSUNG

Denaturiertes Ovalbumin, Serumalbumin, Serumglobulin und Laktoglobulin geben mit Diazobenzolsulfosäure und mit FOLIN's Phenolreagens stärkere Farbreaktionen als die gleichen Proteine im nativen Zustand. Die schwächere Farbreaktion der nativen Proteine wird auf sterische Hinderung durch ihre eng gefalteten Peptidketten zurückgeführt.

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