BBA 75869

THE INADEQUACY OF SODIUM DODECYL SULFATE AS A DISSOCIATIVE AGENT FOR BRAIN PROTEINS AND GLYCOPROTEINS*

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(Received October 25th, 1971)

SUMMARY

Sodium dodecyl sulfate and mercaptoethanol quantitatively solubilized delipidated bovine brain proteins and glycoproteins. Chromatography on Sepharose 4-B gave three distinct peaks. Each of the peaks were identical with respect to their amino acid composition and disc-gel electrophoresis pattern. When one of the peaks was rechromatographed on the same column it redistributed to give the same three peaks, each of which were identical by amino acid composition and disc-gel pattern. It is concluded that sodium dodecyl sulfate can solubilize but not dissociate brain proteins and glycoproteins. It is suggested that membrane proteins and other water-insoluble proteins may drastically differ from water-soluble proteins in their resistance to dissociating reagents and that molecular weights of membrane proteins obtained by sodium dodecyl sulfate electrophoresis may give erroneous results.

INTRODUCTION

With the exception of the S-100 protein[2,3], tubulin[4,5], and two myelin proteins, proteolipid[6,7], and the basic A1 protein[8–12,24], very little is known about the structure of brain proteins and glycoproteins. This is due mainly to their insolubility in aqueous as well as organic solvents. The detergent sodium dodecyl sulfate, in conjunction with mercaptoethanol, is widely used for the denaturation of proteins and dissociation of oligomeric structures[13]. Considerable experimental evidence indicates that nearly all proteins bind at least their own weight of this detergent and exist in solution as linear random coils[14–16]. Electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate and mercaptoethanol has proven to be an extremely useful and reliable method for the determination of molecular weights[17–19]. It seemed, therefore that this detergent might be effective for the solubilization and fractionation of brain proteins and glycoproteins. In this paper, it is shown that while sodium dodecyl sulfate quantitatively solubilizes brain proteins, it does not dissociate them.

* Brain Glycoproteins. II. The preceding paper in this series is ref. 1.

EXPERIMENTAL

Acetone-dehydrated, lipid-extracted bovine brain protein residue was prepared as previously described[1]. Sodium dodecyl sulfate was obtained from BioRad Laboratories. Sepharose 4-B was a product of Pharmacia. Polyacrylamide-gel disc electrophoresis was performed by the procedure described by Weber and Osborn[18] using the normal amount of cross-linker. Amino acid analyses were carried out as previously described[20]. Fractions obtained by Sepharose chromatography for analysis were lyophilized and extracted with acetone to remove sodium dodecyl sulfate and then dried with diethyl ether. Sepharose fractions that were to be rechromatographed on Sepharose were lyophilized and redissolved in 10 ml of water.

RESULTS AND DISCUSSION

Sodium dodecyl sulfate, in the presence of mercaptoethanol, completely solubilized acetone-dehydrated, delipidated bovine brain powder within 24 h at room temperature (as described in the legend to Fig. 1). When the solution was diluted with water 5-fold and centrifuged (100000 × *g*, 4 h) no precipitate was observed. In addition, the colorimetric protein values[21] were unchanged before and after centrifugation. When the undiluted solution was chromatographed on Sepharose 4-B (see Fig. 1) three distinct peaks were obtained in a yield of 92–94 %. However, each of the peaks had identical amino acid compositions (see Table I) and identical disc-gel electrophoresis patterns (see Fig. 3). When Peak 2 (see Fig. 1) was rechromato-

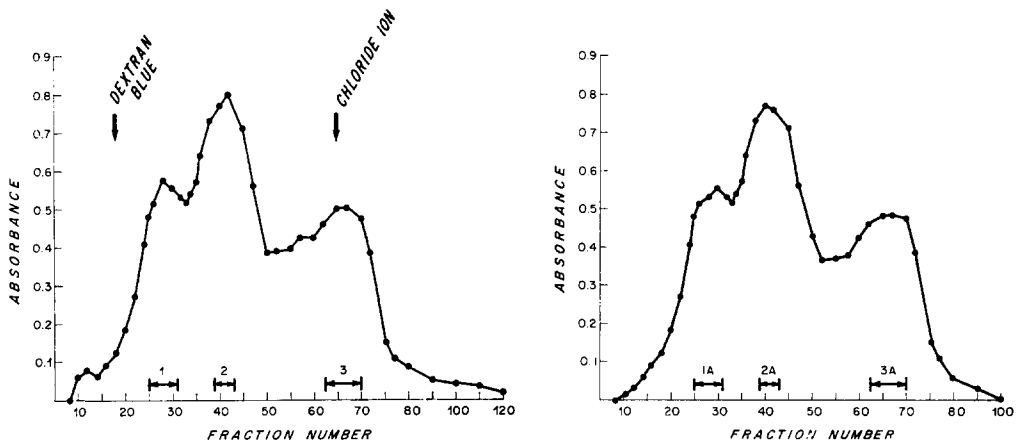

Fig. 1. Chromatography of sodium dodecyl sulfate-solubilized brain residue on Sepharose 4B. Bovine brain residue (200 mg) was stirred for 24 h at 25° in 10 ml of 5 % sodium dodecyl sulfate and 1 % mercaptoethanol. The clear solution was then chromatographed on a column (2.9 cm × 50 cm) of Sepharose 4-B, eluting with 0.1 % sodium dodecyl sulfate and 0.01 % mercaptoethanol. Aliquots (5.0 ml) were collected and analyzed for protein[21]. Portions of Peaks 1, 2, and 3 (indicated by the arrows) were used for amino acid analyses (see Table I) and disc electrophoresis (see Fig. 3). The balance of Peak 2 (Tubes 35–50) was rechromatographed on the same column, as shown in Fig. 2.

Fig. 2. Rechromatography of Peak 2 (Tubes 35–50, see Fig. 1) on the Sepharose 4B column described in Fig. 1. Portions of Fractions 1A, 2A, and 3A (see arrows above) were used for amino acid analyses (see Table I) and disc electrophoresis (see Fig. 3).

graphed on the same column, it redistributed to give the same three peaks (see Fig. 2). Again, each of these three peaks was identical on the basis of amino acid compositions (see Table I) and disc-gel electrophoresis patterns. These results suggest that the brain proteins, when dissolved in sodium dodecyl sulfate and mercaptoethanol, exist in randomly associated form rather than as dissociated polypeptide chains.

TABLE I

AMINO ACID ANALYSES OF PEAKS 1, 2 AND 3 (Fig. 1) AND PEAKS 1A, 2A AND 3A (Fig. 2) GIVEN IN RESIDUES PER 1000 RESIDUES

Amino acid	*Peak:* 1	2	3	1A	2A	3A
Asp	101	103	101	101	99	100
Thr	55	54	53	54	54	56
Ser	68	68	67	65	66	66
Glu	118	119	115	120	116	115
Pro	58	58	57	59	60	60
Gly	79	80	80	79	79	79
Ala	87	89	91	88	88	89
Cys*	13	13	14	15	14	13
Val	60	57	58	58	58	57
Met**	12	12	13	12	12	12
Iso	44	42	44	44	43	44
Leu	90	88	89	91	90	89
Tyr	27	27	28	29	27	27
Phe	43	39	42	40	40	41
Lys	65	66	65	65	65	65
His	23	26	24	24	24	23
Arg	57	59	59	58	57	57

* Including cysteic acid.
** Including methionone sulfoxides.

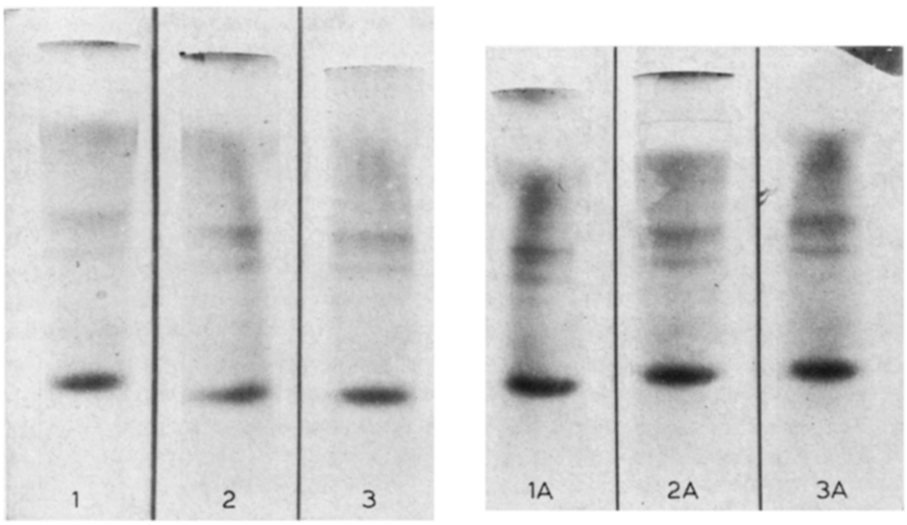

Fig. 3. Polyacrylamide-gel disc electrophoretic patterns of Peaks 1, 2, and 3 (Fig. 1) and Peaks 1A, 2A, 3A (Fig. 2).

The nature of the interaction between sodium dodecyl sulfate and globular proteins such as serum albumin, ovalbumin and chymotrypsinogen[14,15,22] has been intensively investigated as a model for the interaction of the two major chemical components of biological membranes, lipid and protein. However, all of the model proteins that have been examined[14,15,22] are soluble in aqueous solvents. The data presented in this paper as well as recent data presented by Maddy and Kelly[23] suggest that water-soluble proteins may drastically differ from water-insoluble or membrane proteins in their resistance to dissociating reagents.

Of more immediate and practical importance is the observation that molecular weight determinations of water insoluble proteins by sodium dodecyl sulfate–polyacrylamide-gel disc electrophoresis[17–19] may give erroneous results, since the method is based on the assumption that the polypeptide will be completely dissociated in the presence of sodium dodecyl sulfate.

Attempts are presently being made in this laboratory to find an effective dissociative reagent for brain proteins and glycoproteins.

ACKNOWLEDGEMENTS

I thank Dr. David Sakura for helpful discussions. This research was supported by U.S. Public Health Service Grants NS-09616 and FR-05484.

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