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CHARACTERIZATION OF PROTEINACEOUS MATERIAL FROM POSTMORTEM HUMAN BRAIN BY DIFFERENTIAL SCANNING CALORIMETRY

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ABSTRACT

Differential scanning calorimetry, DSC, has been used to investigate the state of water in isolated insoluble proteinaceous fractions from brain tissue of young humans, old humans and Alzheimer diseased cases. It was found that the amount of non-freezable water reaches the highest values in the young samples. Samples from the old age group contain less non-freezable water, which has been interpreted as an indication of age-related changes in tissue proteins. The detected changes can be produced artificially by crosslinking the proteins. The Alzheimer samples, although from an old age group, show some resemblance to young samples. The amount of isolated insoluble material is the same, within experimental error, in all cases.

INTRODUCTION

The characterization of insoluble proteins from postmortem human brain may be useful in identifying structural and conformational changes as well as molecular defects associated with aging and with certain neurologic diseases. It is often assumed that progressive and irreversible accumulation of crosslinked insoluble polymers is one of the factors involved in the process of aging [1]. It has also been assumed that one neuropathological distinction between normal human brain aging and Alzheimer's disease is quantitative with larger deposits of rigid polymers in the latter case [2]. Findings that indicate a decrease in the protein synthesis in Alzheimer's disease have been reported as well [3]. Alterations in the quantities and the thermodynamic behaviour of the high molecular weight brain proteins should reflect these types of structural differences.

In aqueous systems the physical properties and conformation of polymers having hydrophilic groups is largely determined by the water associated with

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the polymer [4]. Associated water exhibits properties different from those of normal pure water. The associated water has been categorized into free water, freezable bound water and non-freezable bound water [5]. Crosslinking and branching affect the freezing, melting and boiling of such water and are related to the amount of non-freezable water in the polymer : water complex [6,7]. The amount of non-freezable water can be estimated by DSC.

The DSC technique has been used to study the interaction of water with different proteins [8–13], with muscle cells [14,15], as well as with food stuffs [16]. The existence of water which cannot be solidified into ice, however low the temperature, in biological materials has been demonstrated beyond doubt. It is generally assumed that this behaviour is due to interactions with the solutes or insoluble solids. In this study we report the results of DSC measurements of water associated with insoluble proteinaceous material from human brain tissue.

EXPERIMENTAL

Materials

Frozen autopsy samples of grey matter from the frontal cortex of fourteen human brains were analysed. Of these twelve were non-neurologic cases in the ages 17–30 years (6 cases) and 60–80 years (6 cases). Two posthumously diagnosed Alzheimer cases of ages 63 and 75 years with no other immediate cause of death were also analysed. Frozen, untreated 5–10 g samples were finely cut and extracted extensively with dimethylsulfoxide at room temperature. The samples were washed with water and dried in vacuum at 323 K and stored at 255 K. From the Alzheimer diseased tissues two samples of each were prepared by similar extraction in order to establish variations in the washing method. The yield of isolated proteinaceous material was $10 \pm 3\%$ of original tissue.

Crosslinking tests

Proteinaceous material isolated from young brains was chemically crosslinked with glutaric aldehyde and with potassium persulfate, respectively.

(1) Three samples (100 mg each) were swelled in buffer solution (pH 7). Different amounts of glutaric aldehyde were added, the aldehyde concentration varying from 2 to 25%. The samples were allowed to react at room temperature for times varying from 10 min to 6 h. The material was washed with water and dried in vacuum at 323 K.

(2) Two samples (100 mg each) were swelled in 5 ml of distilled water. After deaeration with nitrogen 50 mg of potassium persulfate was added and the sample kept at 333–343 K for 20 min, washed and dried as above.

DSC measurements

The measurements were performed with a Perkin-Elmer DSC 1B scanning calorimeter in the temperature range 223–293 K. Samples of weighed amounts of proteinaceous material and water were kept in sealed sample pans at 278 K for 24 h before running the DSC. The samples were cooled to 223 K in the sample compartment and kept at this temperature for 5–10 min and then heated to room temperature. The heating rate was 4–8 K min^{-1}. The enthalpy of melting of water in the samples was compared to that of pure water, and the amount of freezable water in the macromolecular structure was estimated from the area of the melting peak. The lids of the sample pans were then perforated and the samples were dried by heating to 423 K. The exact amount of proteinaceous material in the samples was determined by weighing the pans at intervals. The amount of non-freezable water was calculated as the difference between the total amount of water and the freezable water.

RESULTS AND DISCUSSION

The effect of storage on the brain samples was checked by treating samples from the same brain after varying periods of storage at 255 K. No changes with time could be detected in the samples by DSC; this is consistent with previous findings [16]. The isolated matter was virtually insoluble in water, inorganic and organic solvents and evidently had a very high molecular weight. We found that the amount of isolated material was the same within limits of error for all the samples.

Figures 1–3 show the results of the thermodynamic measurements, i.e., the dependence of the amount of non-freezable water on the amount of proteinaceous material in the aqueous samples. In spite of the noticable variation, there is a clear difference between young and old samples. With a protein content around 10 wt% in the sample the amount of non-freezable water reaches a value around 1.5 g/1 g protein in samples from the young age group and 1 g/g in samples from the old age group. At a concentration of 20 wt% polymer in the sample the average amount of non-freezable water in young and old samples is 1.1 g/g and 0.7 g/g, respectively. In samples from young brains there is an almost linear increase in the amount of non-freezable water with decreasing protein content. In samples from old age brains the amount of non-freezable water remains constant at around 0.5 g/1 g protein in the concentration range 35–60 wt%. Evidently the complicated macromolecular samples from old brains have a structure with lower entropy of conformation than the samples from young brains. This may be due to a greater degree of crosslinking and branching in the old age samples. In model systems it has been shown that in extensively gelled

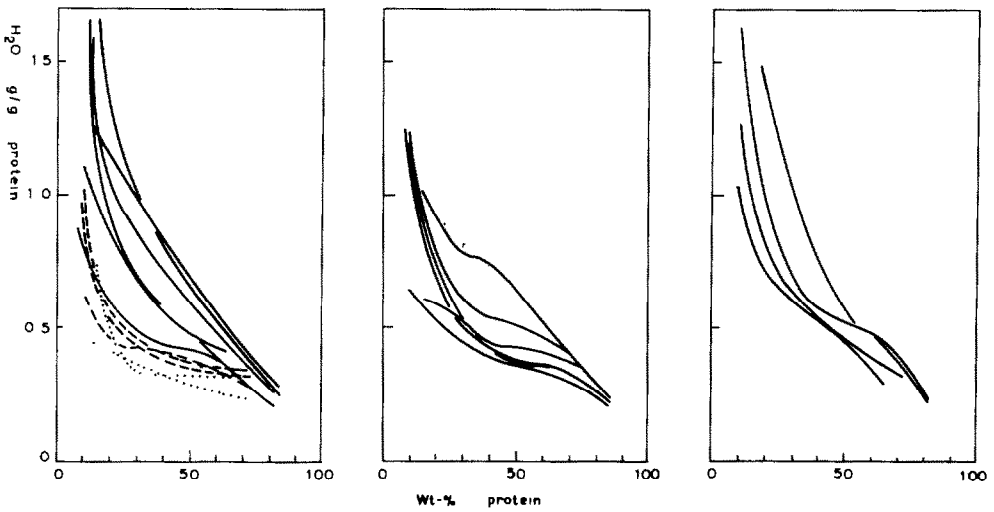

Fig. 1. Amount of non-freezable water associated with isolated insoluble protein from postmortem brain of young humans (———), and from young samples treated with glutaric aldehyde (crosslinking test 1) (- - - - - -) and with potassium persulfate (crosslinking test 2) (· · · · · ·).

Fig. 2. Amount of non-freezable water associated with isolated insoluble protein from postmortem brains of old humans.

Fig. 3. Amount of non-freezable water associated with isolated insoluble protein from postmortem brains of humans deceased from Alzheimer's disease.

samples (crosslinked and branched) there is conformational stabilization by smaller amounts of non-freezable water than in partly gelled samples [6].

Proteinaceous material from Alzheimer cases show some features typical of samples from young brains although from an old age group. The limiting amount of non-freezable water is very high. Samples from young brains and from Alzheimer diseased brains have a structure which expands easily in water. Furthermore, they both contain a considerable portion of material dispersive in water, observed macroscopically as a colloid forming in aqueous samples. On the other hand, conformational stabilization at a concentration of 0.5 g non-freezable water/g protein between 40 and 60 wt% protein is seen in the diagram of Alzheimer brain proteins, thus resembling the samples from the old age group. It is interesting to note that in a study of single muscle fibres [14] the authors report a constant amount of non-freezable water, 0.6 g/g protein, in the range of 20–40% protein in the sample.

In order to establish whether the changes with age in the dynamics of the proteinaceous material can be due to the formation of chemical crosslinks and branches, protein samples from young brains were treated with potassium persulfate and with glutaric aldehyde, respectively. Both are well known crosslinkers [17]. The amount of non-freezable water in the samples

decreased in all cases by this treatment. With the experimental parameters adjusted as described in the experimental section similar thermodynamic behaviour as in the brain proteins from the old age group was achieved. Results from these DSC determinations are marked with dotted lines in Fig. 1.

We conclude that chemical crosslinking and branching is involved in the age related changes of the brain proteinaceous material. The samples from Alzheimer brains probably contain a portion of low molecular weight material absent in the old age samples. We could not find excessive accumulation of insoluble proteins either in the Alzheimer samples or in the old age samples.

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