CONFORMATIONAL TRANSITION OF A MYELIN PROTEIN

John ANTHONY and M.A. MOSCARELLO
Department of Biochemistry, Research Institute, The Hospital for Sick Children, Toronto, Ontario

Received 19 April 1971
Original figures received 25 May 1971

1. Introduction

An interesting transition from conformation to β-structure has been reported for phosvitin [1, 2]. Taborsky [1] found that the transition from random to β-structure was induced by low pH (1.8) and was reversed by raising the pH. Perlman and Grizzuti [2] found that at pH 2.0 phosvitin had a β-structure, while in the pH range 6.0–10.0 the ORD and CD spectra were characteristic of an unordered conformation. The effect of some environmental factors on the transition was also studied.

In a previous study we have described the isolation and some properties of a myelin protein [3]. In initial ORD/CD studies the conformation was found to be α-helical. In an extension of these studies the conformation was found to depend upon the method of preparation. Thus, one procedure yielded the α-helical conformation while the other yielded a β-type structure. This transition could be reversed when the β-form was subjected to conditions which normally yielded the α-helical conformation and vice versa.

In this communication we report the method of preparation of the two forms as well as the effect of some environmental factors such as pH, temperature and ionic strength.

2. Materials and methods

The protein, called N-2, was prepared from myelin by a procedure involving extraction with mercaptoethanol followed by 0.2 N H_2SO_4. The residue was dissolved in chloroform–methanol (1:1) containing 5% 0.1 N HCl and fractionated on a Sephadex LH-20 column [3]. The protein yielded a single component in polyacrylamide gel electrophoresis. It contained small amounts of fucose and hexosamine but no glucose, galactose or mannose. About 2% fatty acid was present in the absence of glycerol, sphingosine and phosphorus.

3. Analytical methods

Protein was determined by the method of Lowry [4]. Polyacrylamide gel electrophoresis was performed by the method of Takayama et al. [5]. Optical rotary dispersion and circular dichroism were done on a JASCO ORD/CD (Durrum Instruments, California, U.S.A.). The results of ORD are expressed as mean residue rotation $[M]$ and CD as mean residue ellipticity $[\theta]$. Cells of path length 0.1 and 1.0 mm were used.

4. Results

4.1. *Preparation of α-helical conformation (procedure I)*

Ten mg of N-2 were dissolved in 2 ml of phenol–acetic acid–water (3:1:1) containing 2 M urea. The solution was placed into a dialysis bag and dialysed against 50% acetic followed by 25, 10 and 5% acetic acid successively and then water, the whole procedure taking 2–3 days. An ultraviolet absorption spectrum of the aqueous solution gave a λ_{max} at 278 nm. The CD spectrum for this protein is shown in fig. 1A. Two minima are observed, one at 210 nm, the other at 220 nm and a crossover point at 202 nm.

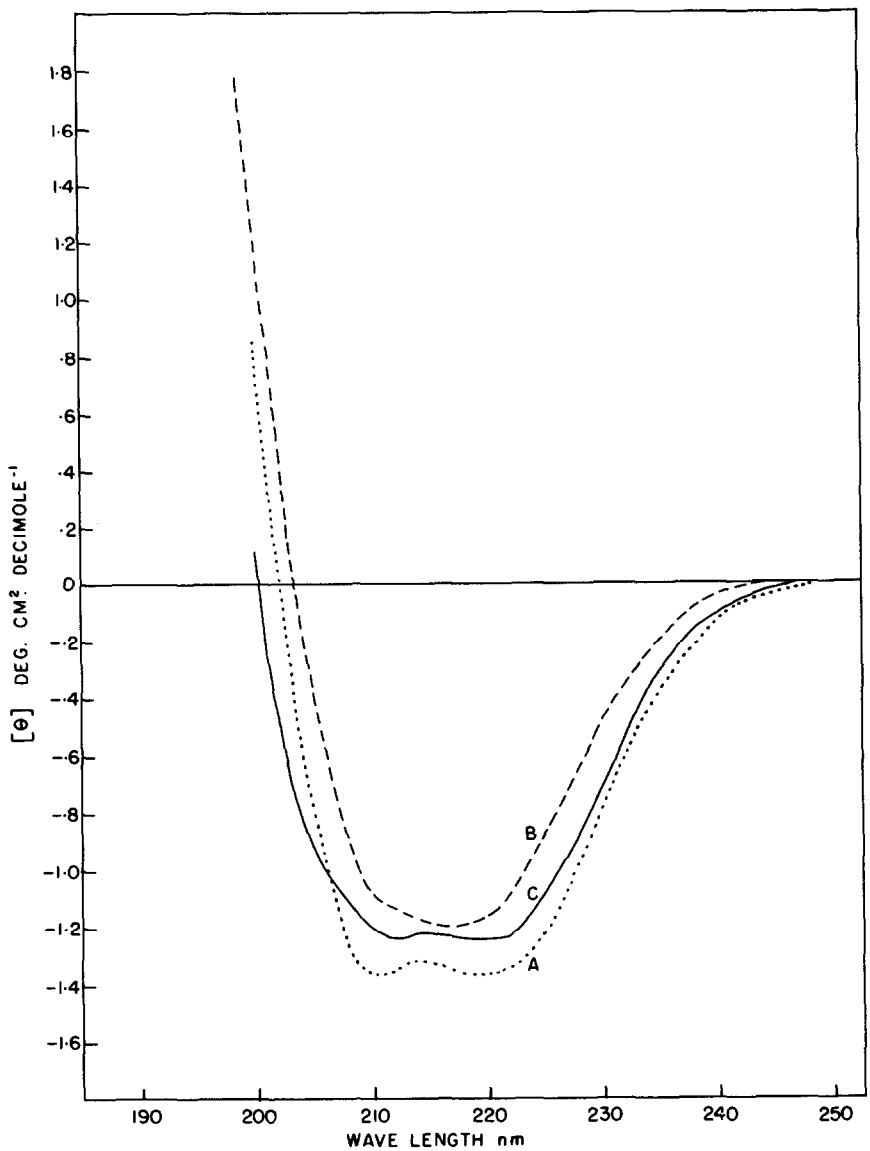

Fig. 1. Circular dichroism of N-2. Curve A: α-helical form resulting from procedure I; curve B: β-form resulting from procedure II; curve C: β-form recycled through procedure I.

The curve is similar to the generalized CD curve for an α-helical homopolypeptide described by Gratzer and Cowburn [6].

4.2. *Preparation of β-structure (procedure II)*

Ten mg of N-2 were dissolved in 2 ml of phenol–acetic acid–water containing 2 M urea as described above and placed into a dialysis sac. The solution was dialysed against 2 M urea, pH 6.0 in the first step followed by 25, 10, 5% acetic and water. Again, the whole procedure took 2–3 days. A typical CD spectrum is shown in fig. 1B. This curve shows a single minimum at 216–217 nm and a crossover at 203–204 nm, similar to the generalized CD curve for a

β-conformational homopolypeptide reported by Gratzer and Cowburn [6].

Transition from β-structure to α-helix was effected by lyophilizing the above solution and putting the dried protein through procedure I. The CD spectrum is shown in fig. 1C. This curve is similar in shape to that of fig. 1A. Two minima are present at 211–212 nm and 220–221 nm.

The transition from α-helix to β-structure can be achieved by simply placing the solution obtained by procedure I in a dialysis sac and dialysing against 2 M urea and then following procedure II. A single minimum was observed at 216–217 nm and a crossover at 203–204.

When we attempted to fit the curves shown in fig. 1 to the computed spectra of poly-L-lysine calculated by Greenfield and Fasman [7], the amount of each structure present could be estimated. The α-helical form contained between 50–70% α-helix while the β-form contained 60–80% β-conformation.

A number of studies were undertaken to determine if disulphide bonds had an effect on the transition. When the protein was reduced with mercaptoethanol and alkylated with iodoacetamide and then some put through procedure I and some through procedure II, only the α-helical form was obtained. Procedure II did not yield the β-conformation. However, performic acid oxidation of the disulphides had no effect on the transition, i.e. performic acid oxidized protein yielded the α-helical form when treated as in procedure I and the β structure when treated as in procedure II. Addition of mercaptoethanol to either α-helical or β-structure did not influence the conformation. When mercaptoethanol was present throughout procedure I or II, only the expected forms were obtained.

The effect of some environmental factors including pH, temperature, ionic strength on the transition were studied next. None of these affected the conformation. The pH of the solution was varied from 1.5 to 6.0 by the addition of dilute HCl. The position of first minimum of the CD curves of the α-helical form varied from 212 nm at pH 1.5 to 209 nm at pH 6.0 while the second minimum varied from 222–220 nm. The crossover at all pH values was at 203–204 nm. No variation in either through position or crossover point was observed at any of the pH values for the β-conformation.

At the various pHs used, a portion of each solution was heated at 50° for 15 min. No effect on the conformation was observed for either the α-helical or β-types.

The ionic strength was varied by adding sodium chloride to the solutions. No effect on conformation was observed at ionic strengths of 0.01–0.04. At higher ionic strengths precipitation of the protein occurred.

4.3. *Polyacrylamide gel electrophoresis*

The two forms were prepared by procedures I and II respectively and applied to polyacrylamide gels as described in Methods. After the electrophoresis was complete the gels were stained with amido black and destained by washing in 7% acetic acid. The scans obtained from these gels are shown in fig. 2. The α-helical and β-forms are shown in fig. 2A and B respectively. Whereas the α-helical form has migrated a reasonable distance into the gel, the β-form moved just beyond the surface of the gel. The different electrophoretic behaviour could be explained by differences in size, the β-form being considerably larger than the α form. $s_{20,w}$ values were found to be 5.9 for the α-form and 19.3 for the β.

5. Discussion

The conclusion that a reversible transition between the α-helical conformation and the β-conformation of a myelin protein is based primarily on the data obtained with circular dichroism. The general shapes of our curves and the λ minimum and λ maximum values are within the range described by Gratzer and Cowburn [6] for homopolypeptides with the respective conformations. The polyacrylamide gel data support this conclusion.

In a recent publication [8], Boos and Gordon observed that galactose-binding protein from *E. coli* separated as 2 components on polyacrylamide gels. However, if the protein was treated with 8 M urea prior to application to the gel one of the two forms was favored. They concluded that two conformational states of the same protein were in equilibrium, since removal of urea resulted in the reappearance of the original gel pattern. In our experiments, exposure of the protein to dialysis against 2 M urea had the effect of favoring one of the two forms, the β-con-

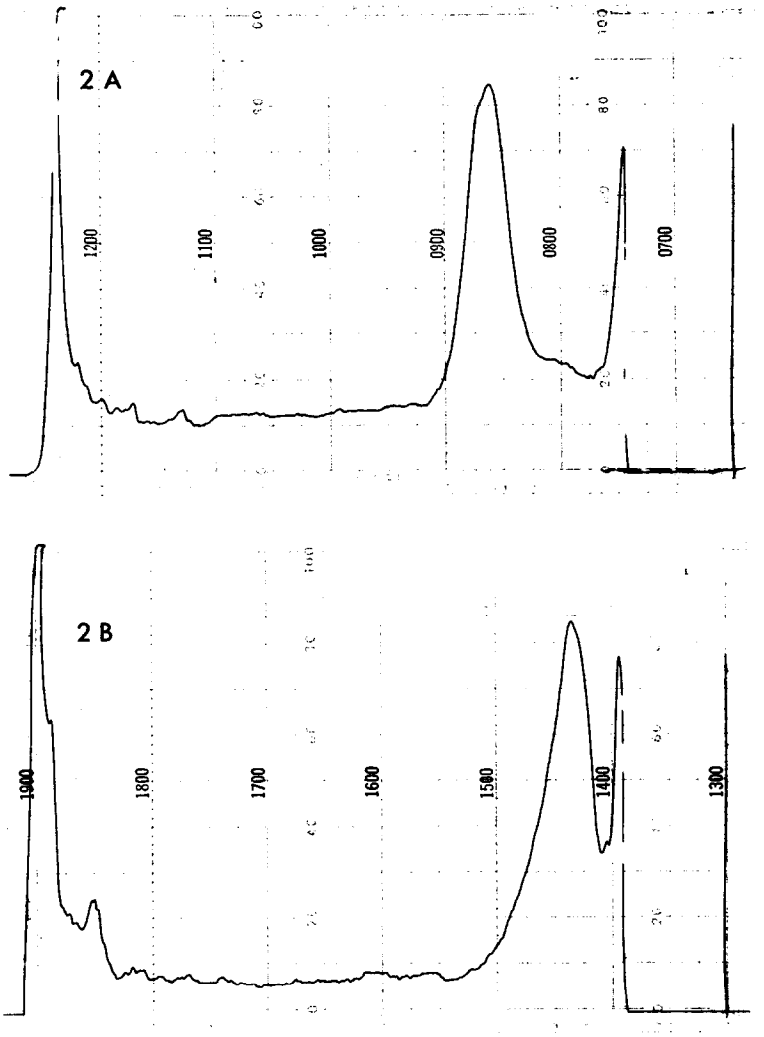

Fig. 2. Polyacrylamide gel electrophoresis. Densitometer scan (A) α-helical form, (B) β-form.

formation. However, the conformational state could be reversed by dialysing the protein by the alternate procedure. When the synthetic polypeptide poly-L-lysine was used, only the random conformation was obtained when it was prepared by either procedure I or II. Similarly, no conformational transition could be demonstrated for the acid-soluble encephalitogen from myelin. Thus, it appears that the transition is not only a function of the procedure but may also be related to some properties of the protein itself.

It is too early to assign a functional significance to this transition. However, it is tempting to speculate that this could be related to the stability of the myelin membrane.

Acknowledgement

The authors gratefully acknowledge the support provided by the Multiple Sclerosis Society of Canada.

References

[1] G. Taborsky, J. Biol. Chem. 243 (1968) 6014.
[2] G.E. Perlman and K. Grizzuti, Biochemistry 10 (1971) 258.
[3] J. Gagnon, P.R. Finch, D.D. Wood and M.A. Moscarello, submitted for publication.
[4] O.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[5] K. Takayama, D.H. MacLennan, A. Tzagoloff and D.C. Stoner, Arch. Biochem. Biophys. 114 (1966) 223.
[6] W.B. Gratzer and D.A. Cowburn, Nature 222 (1969) 426.
[7] N. Greenfield and G.D. Fasman, Biochemistry 8 (1969) 4108.
[8] W. Boos and A.S. Gordon, J. Biol. Chem. 246 (1971) 621.