CONFORMATIONAL TRANSITIONS OF THROMBOPLASTIN APOPROTEIN FROM PIG BRAIN

R. M. HOWELL, H. REZVAN and P. M. SCOPES[+]

Department of Biochemistry, Queen Elizabeth College, London W8 7AH and [+]Department of Chemistry, Westfield College, Hampstead, London NW3 7ST, England

Received 19 October 1978

1. Introduction

Little is known of the mechanism whereby the release of thromboplastin (factor III) as a phospholipid–glycoprotein complex from damaged cell membranes leads to the activation of factor VII in the extrinsic pathway of blood coagulation. The interaction with factor VII has a rapid effect on the acceleration of coagulation and although non-enzymic the subsequent activity of factor VII towards factor X is increased 16 000-fold [1]. Both components of the factor III complex are necessary for clotting and the protein is referred to as apoprotein III.

In [2,3] we used circular dichroism (CD) and infra red spectroscopy as sensitive tools to investigate changes in conformation of purified apoprotein III upon relipidation with suitable mixtures of amphipathic lipids, including those which reflect the asymmetric distribution across the bilayer profile of native membranes. These lipids restored its coagulation activity to an optimum level particularly in the presence of 0.25 M NaCl and we were able to show for the first time that the apoprotein must retain some α-helix in the lipid phase.

In an extension of this earlier study we have used CD to examine the flexibility of the apoprotein III structure in solvents of contrasting polarity so that we may determine the conformation it adopts for the recognition and binding of factor VII. The effect of increasing the concentration of NaCl as well as trifluoroethanol (TFE) on the secondary structure of apoprotein III was therefore studied in separate experiments. These systems were chosen to mimic the ionic state in blood and the hydrophobic core of cell membranes so that an environment devoid of phospholipids, would exclude artefacts arising from light scattering.

2. Materials and methods

Apoprotein III was prepared from pig brains essentially as in [4]. The apoprotein yielded a single component by polyacrylamide electrophoresis, at pH 9.0, in 4.5% (w/v) gels [5]. Filtration of the apoprotein through Sepharose-6B indicated mol. wt 350 000–370 000 and when analysed by SDS–polyacrylamide gel electrophoresis [6] the major band corresponded to mol. wt 59 000.

Circular dichroic spectra of freshly isolated apoprotein III were obtained on a Cary 61 spectropolarimeter as in [2]. Measurements were recorded at 23°C in a cell of pathlength 2 mm in 0.05 M Tris/HCl buffer (pH 7). The data are expressed in the spectra as mean residue ellipticity $[\theta']$, in deg. $cm^2.dmol^{-1}$ plotted against wavelengths in the far ultraviolet region from 250–200 nm. A mean residue weight of 127 was calculated from amino acid analysis and protein was determined by the Lowry method [7].

3. Results

The effects of varied mixtures of TFE–H_2O and NaCl solutions of increasing ionic strength on the circular dichroism of apoprotein III in the far ultraviolet region are shown in fig.1 and 2, respectively.

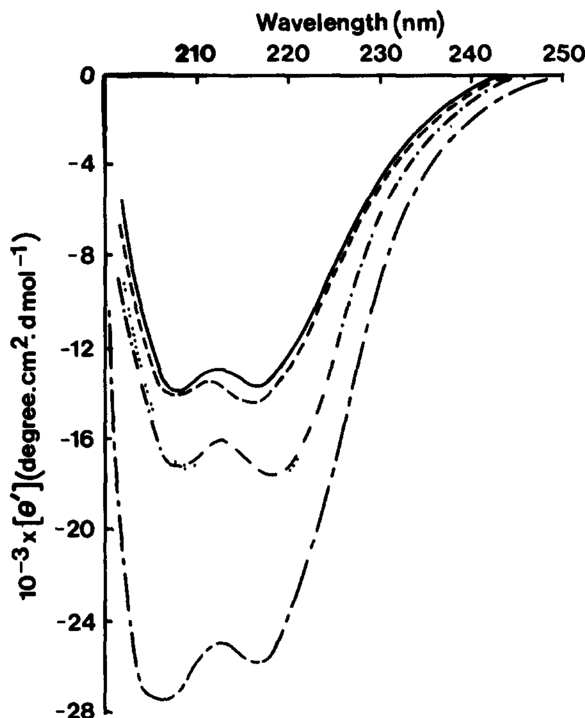

Fig.1. CD spectra of apoprotein III (0 07 mg/ml) in the far ultraviolet (250–200 nm) in mixtures of trifluoroethanol/water at 23°C and buffered at pH 7 0 with 0 025 M Tris/HCl/0 05 M NaCl. TFE/H_2O (0 100, v/v) (——), TFE/H_2O (10 90, v/v) (– – – –), TFE/H_2O (40.60, v/v) (– – –), TFE/H_2O (70 30, v/v) ( ) and TFE/H_2O (90 10, v/v) (– · – – –)

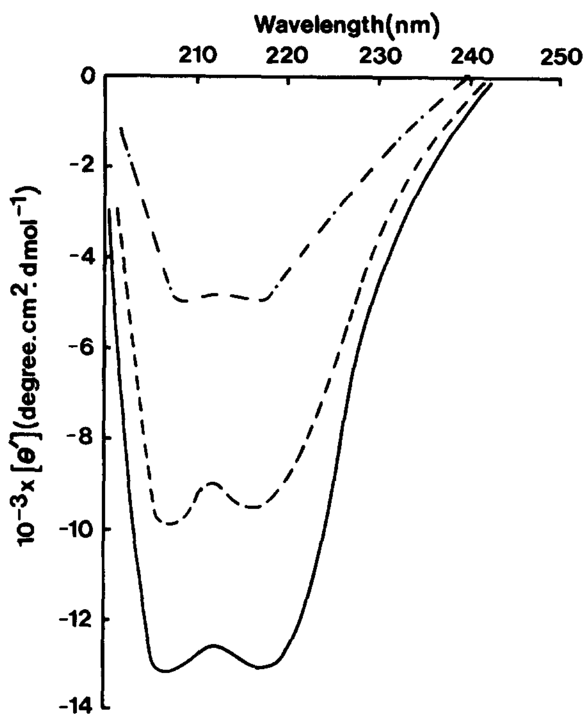

Fig.2 CD spectra of apoprotein III (0 07 mg/ml) in the far ultraviolet region in various concentrations of NaCl 0.05 M (——); 0 25 M (– – –), and 0 5 M (– – –) Conditions were the same as in fig.1

In both solvent systems the spectra are characterized by two negative extrema at 208 nm and 218 nm; the crossover point from negative to positive dichroism being located at 202 nm. These Cotton effects are features of proteins and polyamino acids whose peptide chain is in a right-handed α-helical conformation [8]. The percentage of each secondary structure present was estimated by fitting the curves shown to the computed spectra of poly-(L-lysine) calculated [8] and these data are shown in table 1.

The marked tendency of apoprotein III to form an α-helix is seen in fig.1, when the proportion of TFE relative to H_2O was increased in the buffer. TFE was selected for this study because of the ability of halogenated alcohols to convert to an α-helical form any polypeptide with this potential. Nevertheless, the volume percentage of TFE in H_2O had to reach 40% before the helical-forming effect was apparent, whereupon it increased the α-helix to 40%. It remained at this level in the presence of up to 70% TFE before

Table 1
Percentages of α-helix (α), β-structure (β) and unordered conformation (ρ) of apoprotein III in various solvents calculated by the method in [8] from the CD spectra in fig 1,2

Solvent	%α	%β	%ρ
0 05 M NaCl	27	33	40
0 25 M	30	40	40
0 50 M	3	37	60
H_2O	27	33	40
TFE–H_2O, 10 90(v/v)	27	33	40
TFE–H_2O, 40 60(v/v)	45	18	40
TFE–H_2O, 70 30(v/v)	45	18	40
TFE–H_2O, 90 10(v/v)	80	0	20

the apoprotein underwent a large conformational change in 90% TFE–10% H_2O, which raised the α-helical content to 80% (fig.1, table 1). A further conversion would probably require reduction of the disulphide bonds. It is notable that the β-conformation was not measurable in this amount of TFE and the unordered structure was halved from that present originally.

In contrast to the results in TFE, it can be seen from the spectra in fig.2 that the intensities of the two principal negative extrema are much reduced in the presence of NaCl. Overall, the reduction in ellipticities reflects a large decrease in the α-helical form from 27%, to a very low content of 3%, when salt was raised from 0.05 M to 0.5 M (table 1). At the same time there was no apparent $\alpha \rightarrow \beta$ transition, and the unordered structure was raised by 20%. Careful inspection of both negative extrema revealed that the $[\theta']_{208}/[\theta']_{222}$ ratio was unchanged and this, together with the absence of any spectral shifts suggests that no significant light scattering had occurred [9].

Finally, in the presence of low levels of TFE (10%) and NaCl (0.05 M) the retention of a high degree of helical order (29%), while the H_2O concentration is relatively high, is a sign of a fairly stable helical conformation. The level of 29% of α-helix in apoprotein III changed very little on relipidation [2] even though the present study has shown it to be highly sensitive to extreme changes of polarity (table 1) The significance of the α-helix for binding lipid to this membrane-derived apoprotein probably represents a similar requirement to that shown for plasma lipoprotein [10] and glycophorin [11].

4. Discussion

The reduction in CD ellipticity and, by implication, α-helix when apoprotein III is measured in the presence of increasing concentrations of NaCl probably reflects changes in its secondary structure following an accelerated self-association of freshly isolated material. In the absence of detergent, apoprotein III slowly undergoes spontaneous aggregation and, using Sepharose 6B filtration, we have shown that the monomer associates to give a hexamer.

It appears that the major influence of NaCl on the CD spectra of the self-associated apoprotein III in solution could be due to the clustering of adjacent helices and their transformation, at least partially, into a more extended structure with interchain hydrogen bonds [9]. The compact, but soluble, multimer which results would account for the reduced ellipticity through being less dissymmetric than the monomer. This overall effect of NaCl is compatible with a low ranking in the Hofmeister salt series [11] and its ability to neutralize charge on peptide side-chains and sugar residues.

On the other hand, the most probable explanation for the increased α-helix found in 80% TFE–20% H_2O is the dissociation of the hexameric apoprotein, in a medium of relatively low dielectric constant. This process can be interpreted as bringing about the disruption of hydrophobic-bonded areas between adjacent monomers and their reassembly as helices is encouraged by intramolecular hydrogen bonds [13]. These observations are also consistent with the CD spectra of a variety of polyamino acids and cyclic decapeptides, such as tyrocidines. Their aggregation behaviour in aqueous salt solutions and organic solvents allows them to be used as models for the interpretation of CD measurements arising from changes in the secondary and quaternary structures of natural proteins [14–16].

Although TFE can mimic successfully the α-helical promoting environment encountered in cell membranes the present study has indicated that the CD spectra of apoprotein III in TFE alone may not be representative of its biologically active conformation. As well as providing a hydrophobic environment, it has been suggested that changes in the physical state of phospholipids across a native membrane encourage regions of high protein density [17]. Similarly, disturbance of the normal asymmetric distribution of lipids across the bilayer profile during membrane damage may encourage clotting by inducing an aggregated state, provided that charge neutralization on the protein also occurs in the presence of salts.

In attempting to relate CD spectra to thromboplastin function, following the rearrangement of its components, we conclude that a solution of 0.25 M NaCl in 80% TFE–20% H_2O may provide suitable membrane-like conditions for optical measurements. This solvent system may also be applicable to CD studies on the biologically active conformation of other intrinsic membrane proteins [18].

Acknowledgements

H.R. was in receipt of a Queen Elizabeth College demonstratorship. This work was supported by grants from the British Heart Foundation and the Science Research Council. The authors wish to thank Mrs. Maria Barrett for running the CD spectra.

References

[1] Zur, M and Nemerson, Y. (1978) J Biol Chem 253, 2203–2209.
[2] Howell, R. M and Rezvan, H (1977) Biochem. Soc Trans. 5, 1552–1554
[3] Rezvan, H. and Howell, R M. (1978) Biochem. Soc. Trans. 6, 235–237
[4] Nemerson, Y. and Pitlick, F A (1970) Biochemistry 9, 5105–5110.
[5] Davis, B. S (1964) Ann. NY Acad Sci 121, 404–427
[6] Dunker, A K. and Rueckhart, R. R. (1969) J Biol. Chem. 244, 5074–5080
[7] Lowry, O. H , Rosebrough, N J., Farr, A L and Randall, R J (1951) J Biol Chem. 193, 265–275
[8] Greenfield, N and Fasman, G D. (1969) Biochemistry 8, 4108–4116.
[9] Fasman, G D., Hoving, H and Timasheff, S N (1970) Biochemistry 9, 3311–3324.
[10] Andrews, A. L , Atkinson, D., Barratt, M. D., Finer, E G., Hauser, H , Henry, R , Leslie, R. B , Owen, N. L., Phillips, M. C and Robertson, R. N (1976) Eur. J Biochem. 64, 549–564
[11] Furthmayr, H , Galardy, R. E , Tomita, M and Marchesi, V T (1978) Arch. Biochem. Biophys 185, 21–29.
[12] Von Hippel, P H. and Schleich, T. (1969) in. Structure and Stability of Biological Macromolecules (Timasheff, S N. and Fasman, G. D eds) pp. 417–574, Dekker, New York
[13] Tomimatsu, Y and Galfield, W. (1966) Biopolymers 4, 653–662.
[14] Arfmann, H. A , Labitzke, R. and Wagner, K G (1975) Biopolymers 14, 1349–1381.
[15] Conco, G , Patrone, E and Salaris, F. (1971) Macromolecules 4, 283–288.
[16] Laiken, S., Printz, M. and Craig, L. C. (1969) J. Biol Chem. 244, 4454–4457
[17] Wallace, B. A and Engelman, D M. (1978) Biochim. Biophys. Acta 508, 431–449
[18] Lotan, N. (1974) in Peptides, Polypeptides and Proteins (Blout, E. R. et al. eds) pp 157–165, John Wiley and Sons, New York