DETERMINATION OF SECONDARY STRUCTURES IN ISOLATED OR MEMBRANE PROTEINS BY COMPUTER CURVE-FITTING ANALYSIS OF INFRARED AND CIRCULAR DICHROIC SPECTRA

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1. Introduction

One of the major unsolved problems of the understanding of membrane function is the knowledge of the secondary structure of membrane proteins in various membranes of different functional types or of a distinct membrane type in different functional states. Although several attempts have been made to tackle this problem by the valuation of CD* [1] or IR [2] composite spectra, the task still appears to be far from solution. The main objections to the validity of the results obtained as yet may be summarized as follows.

1) The applied CD basis spectra derived from the three conformations of poly amino acids are not representative for the peptide structures occurring in biomembranes.

2) The CD composite spectra of particulate membrane suspensions are heavily disfigured by optical artifacts.

3) The determination of the proportion of α-structure by the application of an empirical formula to the 222 nm CD band [3] or the estimate of a change in β-structure contents by tracing an absorbancy variation in the 6.05 μm IR band [2] are at best rough approximations to the analysis of composite spectra unconfirmed by independent evidence.

In the present paper, some further methodological approaches to the reduction or elimination of these uncertainties are described. They include the calculation of CD and IR basis spectra from proteins of known structural composition (by X-ray analysis) and their application to a computer curve-fitting analysis of CD and IR composite spectra of soluble and membrane-bound proteins as to the percentage composition of secondary structures.

Abbreviations:

CD: Circular Dichroism; IR: Infrared; α-structure: α-helical structure including 3_{10}-helix; β-structure: parallel and antiparallel pleated sheet structures; ρ-structure: remainder asymmetrical structures.

2. Materials and methods

Lysozyme (C.F. Boehringer, Mannheim), ribonuclease (Serva, Heidelberg), insulin (VEB Berlin-Chemie) and serum albumin (VEB Serumwerk Dessau) were crystalline materials. Metmyoglobin from ox (Biocatalysis Department of this institute) was a lyophilized preparation. The synaptosomal membranes were prepared from pig brain according to the method of Samaha [4]. The materials were dissolved or suspended in D_2O-Tris-buffer (pD = 7.5) 40–45 hr before their spectra were taken. The protein concentration was 30 mg/ml for both the CD spectra (path length 10 μm) and the IR spectra (path length 50 μm).

The CD and IR basis spectra (figs. 1 and 2) were calculated from the CD and IR composite spectra of ribonuclease, lysozyme and metmyoglobin the structural composition of which is known from X-ray analysis; the arithmetical mean of the values obtained by different authors [5] were used. Due to the proximity of their maxima the IR basis spectra of α- and ρ-structure could not be computed separately so that the curve-fitting analysis of IR composite spectra is

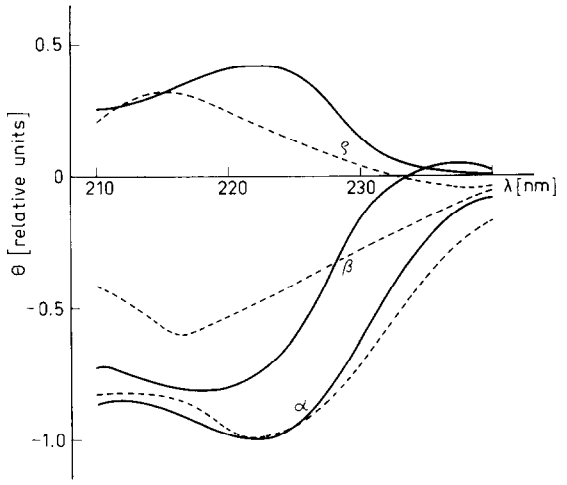

Fig. 1. CD basis spectra for the curve-fitting determination of α-, β- and ρ-structures in dissolved proteins (drawn out lines) calculated from CD composite spectra of proteins of known structural composition (by X-ray analysis) and modified CD basis spectra for the curve-fitting determination of secondary structures in membrane-bound proteins (dotted lines) derives from the original basis spectra to reach a complete fit to the experimental CD composite spectra of membrane suspensions. The curves are normalized on the basis of the 222 nm minimum of the α-structure.

restricted to the determination of the precentages of β- and (α + ρ)-structures [6].

The mathematical procedure for the calculation of the basis spectra and their use for the determination of the structural composition of proteins, using the principle outlined by Saxena and Wetlaufer [5], as well as the critical examination of the methodological prerequisites were published elswhere [7]. The modified CD basis spectra (fig. 1) for the analysis of CD composite membrane spectra were obtained as described earlier [8].

3. Results and discussion

When comparing the results obtained by different approaches (table 1) it should be borne in mind that the figures have but a statistical meaning. Thus, the percentages 24 ± 2.0 or 6 ± 3.0 found for β-structure in serum albumin or in metmyoglobin by the curve-fitting analysis of IR composite spectra mean that the real values lie between 20 and 28 or 0 and 12 per cent. As well-known, X-ray analysis of proteins shows similar variations of the data [5].

Table 1

Percentage of α-, β- and ρ-structures in various proteins as obtained by the computer curve-fitting analysis of CD or IR composite spectra when using calculated CD basis spectra (approach I), calculated IR basis spectra (approach II) or modified CD basis spectra (approach III).

Analyzed material	Approach	α	β	ρ
Insulin	I	42 (± 1.8)	14 (± 1.0)	45 (± 3.6)
	IV	40 – 43	–	–
Serum albumin	I	45 (± 1.1)	11 (± 0.6)	44 (± 2.3)
	II	*)	24 (± 2.0)	*)
Ribonuclease	II	*)	34 (± 0.4)	*)
	IV	12	36	52
Lysozyme	II	*)	10 (± 2.1)	*)
	IV	35	10	55
Metmyglobin	II	*)	6 (± 3.0)	*)
	IV	71	0	29
Synaptosomal membranes	I	19	36	45
	III	24 (± 1.3)	29 (± 4.6)	47 (± 4.3)
	II	*)	32 (± 3.7)	*)

*) $\alpha + \rho = 100 - \beta$.

The number in brackets are the standard deviations. For the sake of comparison, the structural data found by X-ray analysis (approach IV) of insulin [9]; ribonuclease, lysozyme and metmyoglobin (mean values as published in [5]) are also shown.

3.1. *Dissolved proteins*

The application of the CD basis spectra (approach I) or the IR basis spectra (approach II) yields values for the structural composition of the proteins which are in the same range for β-structure in serum albumin and compare well with the data obtained for the secondary structures in the other proteins by X-ray analysis (approach IV). This result appears to corroborate the ap-

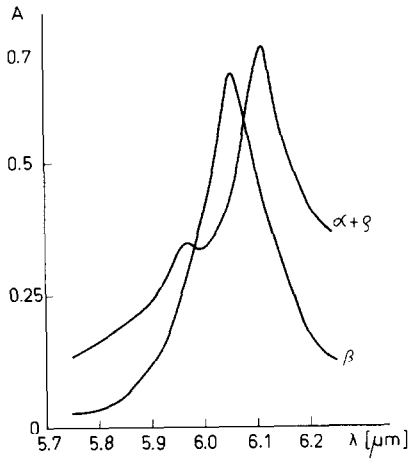

Fig. 2. IR basis spectra for the curve-fitting determination of β- and (α + ρ)-structures in dissolved or membrane-bound proteins calculated from IR composite spectra of proteins of known structural composition (by X-ray analysis).

proaches I and II as acceptable, independent methods for the determination of secondary protein structures.

3.2. *Membrane-bound proteins*

The application of the calculated CD basis spectra to the computer curve-fitting analysis of CD membrane spectra is not seldom possible due to the distortion of the experimental spectra by optical artifacts as discussed by Urry [1]. This difficulty is overcome by the use of modified CD basis spectra considering the distortions (approach III). The feasibility of this approach appears to be corroborated by the excellent agreement between the percentages of β-structure determined by approaches III and II. It must be stressed, however that the applicability of the modified CD basis spectra requires the use of membrane suspensions of distinct properties especially concerning particle size and concentration.

The proportion of α- and ρ-structures as obtained by the analysis of CD spectra of membranes cannot be checked directly by an independent method. However, a comparison of the original and the modified basis spectra (fig. 1) shows that the curves of α- and also of ρ-structures are distorted much less by optical artifacts than the curve of β-structure. Since the value for β-structure obtained by the analysis of CD membrane spectra (approach III) is confirmed by the evaluation of IR membrane spectra undisturbed by optical artifacts, it may be concluded that the percentages of α- and ρ-structures as determined by the analysis of CD composite spectra are also not far from reality.

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References

[1] D.W. Urry, Biochim. Biophys. Acta 265 (1972) 115.
[2] J.M. Graham and D.F.H. Wallach, Biochim. Biophys. Acta 241 (1971) 180.
[3] N. Greenfield and G.D. Fasman 8 (1969) 4108.
[4] F.J. Samaha, J. Neurochem. 14 (1967) 333.
[5] V.P. Saxena and D.B. Wetlaufer, Proc. Natl. Acad. Sci. U.S. 68 (1971) 969.
[6] R. Grosse, J. Malur and K.R.H. Repke, Acta Biol. Med. Germ. 28 (1972) K 11.
[7] R. Grosse, J. Malur, W. Meiske, J.G. Reich and K.R.H. Repke, Acta Biol. Med. Germ., in press.
[8] R. Grosse, J. Malur and K.R.H. Repke, Acta Biol. Med. Germ. 27 (1971) K 25.
[9] Y.-H. Chen and J.T. Yang, Biochem. Biophys. Res. Commun. 44 (1971) 1285.