RIBOSOMAL PROTEINS. SECONDARY STRUCTURE OF INDIVIDUAL RIBOSOMAL PROTEINS OF *E. COLI* STUDIED BY CIRCULAR DICHROISM

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1. Introduction

In the last three years homogenous proteins from *E. coli* ribosomes have been isolated and characterized by chemical, physical and immunological techniques in several laboratories [1–8]. To obtain further insight into the structure and ultimately the function of the ribosome at a molecular level, it is necessary to examine the properties of the individual ribosomal proteins as extensively as possible. This communication reports information obtained for 20 individual ribosomal proteins by circular dichroism which has been frequently used to investigate the secondary structure of proteins. In particular, the question is raised whether all the ribosomal proteins are similar to each other in their secondary structure, whether there are different classes of proteins or whether the proteins all have different structural features.

2. Materials and methods

The individual ribosomal proteins used in this study have been isolated from *E. coli* ribosomes as briefly described elsewhere [8]. Each lyophylized protein (0.5 mg) was dissolved in 50 μl formic acid at 0°, diluted with 10^{-3} M HCl to 250 μg/ml and dialyzed against five changes of 10^{-3} M HCl at 0°. In this way the proteins remain in solution. For exact determination of the concentration, aliquots were hydrolyzed for 20 hr in 6 M HCl at 110° and the amino acid contents were determined in an automatic amino acid analyzer (Bio-Cal, München). The amino acid analyzer was calibrated before and after the analysis. A correction factor of 10% was used for the degradation of threonine and serine in the hydrolysis, for non-hydrolyzed peptide bonds and for those amino acids, e.g. cysteine and tryptophane, which were not determined.

Circular dichroism was measured in a dichrograph CD 185 (Roussel-Jouan) at room temperature with cells of 0.1 cm and 0.5 cm path length. As differences exist which possibly depend on the type of instrument [9] with respect to the standard spectra for α-helix and random coil, the data of Velluz and Legrand [10] for polyglutamic acid were used as standard spectra, because they were also determined with a Roussel-Jouan dichrograph.

3. Results

Fig. 1 shows the circular dichroic spectrum of total ribosomal protein between 270 and 190 nm. $\Delta\epsilon$ is negative down to 195 nm with a shoulder at 220 nm, a minimum at 204 nm and a crossover at 194 nm. This is the spectrum characteristic for a structure containing mostly random coil, a smaller amount of α-helix and possibly β-structures. Fig. 2 shows the spectrum of one representative individual protein. It is quite similar to that of the total proteins. Fig. 3 gives the spectrum of protein CM 84/2. This and protein CM 84/1 are the only ones which differ strongly from all the other proteins and from total ribosomal proteins. Both proteins give a negative double peak at 222 and 208 nm, characteristic for an α-helical structure. None of the ribosomal proteins show effects of aromatic amino acids in the range above 250 nm.

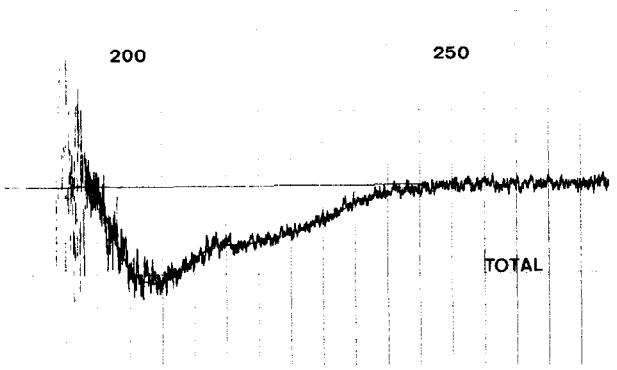

Fig. 1. Circular dichroism of total ribosomal proteins between 190 and 270 nm.

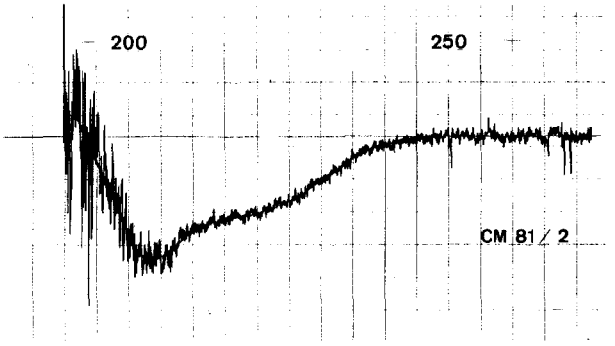

Fig. 2. Circular dichroism of a representative individual ribosomal protein (CM 81/2).

Table 1
α-Helix random coil content of proteins.

Protein	isolated from	acidic or basic	helix content 222 nm	helix content 210 nm	rand. coil content 202 nm	rand. coil content 199 nm
Total	70 S		30	30	55	60
P 29/1	70 S	b	27	32	–	–
P 29/2	70 S	b	22	25	55	70
P 32/2	70 S	b	23	26	65	68
P 33/4	70 S	b	24	29	–	–
CM 57/3	70 S	b	19	26	–	–
CM 60/6	70 S	b	20	24	–	–
CME 4/4	70 S	b	23	23	–	–
CME 5/6	70 S	b	23	24	60	75
CM 81/2	30 S	b	33	32	43	58
CM 81/3	30 S	b	24	27	69	75
CM 81/4a	30 S	a	28	33	60	67
CM 81/6	30 S	b	35	40	43	44
CM 81/8	30 S	b	31	38	66	70
CM 81/13	30 S	b	24	28	73	77
CM 84/1	50 S	a	49	55	45	56
CM 84/2	50 S	a	46	48	45	56
CM 84/5	50 S	b	30	31	53	62
CM 84/9	50 S	b	26	30	63	72
CM 84/10	50 S	b	28	33	69	82
CM 84/13	50 S	b	23	28	73	79

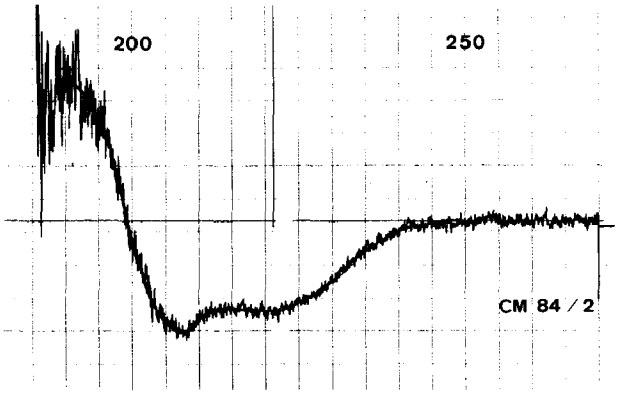

Fig. 3. Circular dichroism of a ribosomal protein (CM 84/2) with high α-helix content.

The α-helical content is frequently estimated from the molar circular dichroism at 222 nm of the n-π^* transition of the carbonyl group. If β-structures are present this estimate can lead to erroneous results. Preferably the n-π^* transition of the amide group polarized parallel to the helical axis should be used. For this reason the molar circular dichroism at 210 nm was measured. At this wave length the value for random coils as well as β-structures is nearly zero [11]. Table 1 gives the helix contents determined at 222 nm uncorrected for β-structures and those determined at 210 nm. Within experimental accuracy these values are in good agreement. In addition, the helical content and the content of random coil as determined at 198 and 202 nm add up to nearly 100%. It therefore seems probable that β-structures are only present to a very small extent.

Since all of the proteins were investigated under conditions under which electrostatic effects of the side groups could effect the conformation of the proteins, the influence of the ionic strength was studied by adding NaF up to a concentration of 0.1 M to some of the protein solutions. Opalescence and the resulting scattering effects make it difficult to evaluate the CD spectra quantitatively, however qualitatively the spectra have the same shape and no red shift of the crossover. It has been shown that the crossover is not effected by two types of scattering [12]. Therefore the electric charges of side groups apparently have little influence on the secondary structure of ribosomal proteins.

4. Discussion

Sarkar et al. [13] and Sarkar and Yang [14] subtracted the optical rotatory dispersion spectrum as well as CD spectra of ribosomal RNA from those of intact ribosomes and they thus obtained a spectrum for ribosomal proteins yielding 25% helix content. The same value was found for the isolated total ribosomal protein. From this agreement they concluded that the secondary structure of the ribosomal proteins was not disturbed by disrupting the ribosomal structure. Cotter and Gratzer [15] compared the position of the amide I band in the infrared spectra of ribosomes and of ribosomal proteins and RNA. They found that the structure of the isolated total ribosomal proteins in acidic solution was equivalent to that of the native proteins in the intact ribosome. Furthermore, they found no indications for β-structures in the intact ribosome.

The results reported here are in agreement with those of Sarkar et al. [13, 14] and of Cotter and Gratzer [15]. The analysis of the CD spectra in this communication leads to a helical content of 30%, both for the total ribosomal protein and for the mean of the isolated individual proteins. The occurrence of β-structures in the isolated proteins under the experimental conditions seems unlikely.

There are three acidic individual proteins (marked *a* in table 1) among the ribosomal proteins of *E. coli*. These are proteins CM 81/4a with a high content of glutamic acid and proteins CM 84/1 and CM 84/2

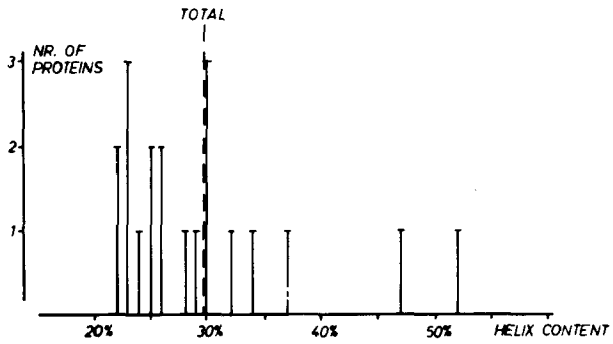

Fig. 4. Number and α-helix content of ribosomal proteins. The abscissa shows the mean value of helix content as derived from 210 nm and 222 nm.

with a high content of alanine and glutamic acid. Protein 81/4a is identical to protein 30S-2 described by Craven et al. [7] and Möller et al. [16]; proteins CM 84/1 and CM 84/2 are identical to the A-proteins isolated by Möller et al. [16] as shown by comparison of the amino acid compositions and by two dimensional polyacrylamide electrophoresis [17].

Proteins CM 84/1 and CM 84/2 with a high alanine content have an α-helical content of 50% (fig. 4) while the α-helical content of all the other proteins ranges between 20 and 40%. The high content of α-helix in the alanine rich proteins is in agreement with a rule of Prothero [18] according to which the α-helical content depends primarily on the proportion of alanine and leucine. The structural difference between proteins CM 84/1 and CM 84/2 on one hand and the other ribosomal proteins on the other hand together with the finding [19] that antibodies against these proteins inhibit phenylalanine incorporation in the poly U system much more strongly than antibodies against other individual ribosomal proteins suggest a distinct and important role of these proteins.

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References

[1] W.Möller and J.Widdowson, J. Mol. Biol. 24 (1967) 367.
[2] R.R.Traut, P.B.Moore, H.Delius, H.Noller and A.Tissières, Proc. Natl. Acad. Sci. U.S. 57 (1967) 1294.
[3] E.Kaltschmidt, M.Dzionara, D.Donner and H.G.Wittmann, Mol. Gen. Genet. 100 (1967) 364.
[4] S.Fogel and P.S.Sypherd, Proc. Natl. Acad. Sci. U.S. 59 (1968) 1329.
[5] P.B.Moore, R.R.Traut, H.Noller, P.Pearson and H.Delius, J. Mol. Biol. 31 (1968) 441.
[6] S.J.S.Hardy, C.G.Kurland, P.Voynow and G.Mora, Biochemistry 8 (1969) 2897.
[7] G.R.Craven, P.Voynow, S.J.S.Hardy and C.G.Kurland, Biochemistry 8 (1969) 2906.
[8] H.G.Wittmann, G.Stöffler, E.Kaltschmidt, V.Rudloff, H.G.Janda, M.Dzionara, D.Donner, K.Nierhaus, M.Cech, I.Hindennach and B.Wittmann, FEBS Sympos. 21 (1970) 33.
[9] S.Beychok, Ann. Rev. Biochem. 37 (1968) 437.
[10] L.Velluz and M.Legrand, Angew. Chemie 77 (1965) 842.
[11] W.B.Gratzer and D.A.Cowburn, Nature 222 (1969) 426.
[12] D.W.Urry and T.H.Ji, Arch. Biochem. Biophys. 128 (1968) 802.
[13] P.K.Sarkar, J.T.Yang and P.Doty, Biopolymers 5 (1967) 1.
[14] P.K.Sarkar and J.T.Yang, in: Conformation of Biopolymers, ed. G.N.Ramachandran (Academic Press, London, New York, 1967).
[15] R.J.Cotter and W.B.Gratzer, European J. Biochem. 8 (1969) 352.
[16] W.Möller, H.Castleman and C.P.Terhorst, FEBS Letters 8 (1970) MS 866.
[17] E.Kaltschmidt and H.G.Wittmann, Anal. Biochemistry, in press.
[18] J.W.Prothero, Biophys. J. 8 (1968) 1236.
[19] G.Stöffler and H.G.Wittmann, to be published.