SALT-INDUCED CONFORMATIONAL CHANGES IN, AND SECONDARY STRUCTURE ANALYSIS OF, HALOPHILE RIBOSOMAL 'A' PROTEINS EQUIVALENT TO EL7/EL12

G. E. WILLICK, R. E. WILLIAMS and A. T. MATHESON+

Division of Biological Sciences, National Research Council of Canada, Ottawa, K1A OR6 Canada

Received 24 October 1977

1. Introduction

An acidic protein present in the 50 S subunits of *E. coli* was first reported [1]. This protein exists as a dimer, and is characteristically present in multiple copies [1]. The equivalent protein, L12 or its acetylated form, L7, is found in other organisms. The complexity of the ribosome, and the necessity of retaining a well-defined function in protein biosynthesis, suggest that functionally equivalent proteins will retain their secondary and tertiary structure despite changes in the primary structure. In order to test this hypothesis, we have compared the secondary structure, as measured by the CD, of the acidic proteins from three bacteria which have some differences in their primary structure. In two cases, the mesophile *E. coli* and an unidentified moderate halophile (NRCC 41227) [2], the complete primary sequence of the A protein is known [3,4], while in the case of the extreme halophile *Halobacterium cutiribrum* about two-thirds of the sequence of the acidic protein, thought to be equivalent to EL12, is known ([5], Oda, G., unpublished data).

2. Materials and methods

The acidic proteins from *E. coli* and the moderate halophile (grown in media containing 0.5 M NaCl) were prepared by the method in [6], and that from *H. Cutiribrum* by that in [7]. Proteins were dissolved in 0.1 M potassium chloride, 2 mM potassium phosphate buffer, and dialyzed overnight. Additional potassium chloride or trifluoroethanol was added to the above buffer prior to use. Circular dichroism experiments were performed with a Cary 61 circular dichrometer at 27°C. Protein concentrations were determined by amino acid analyses. CD spectra were analyzed for secondary structure by a linear least squares procedure [8], using the reference spectra [9].

3. Results and discussion

The CD spectra of *E. coli* L12 in 0.1 M and 3.0 M potassium chloride and in 75% trifluoroethanol are show in fig.1. The spectrum exhibits a real, but small, increase in ellipticity as the salt concentration is increased, corresponding to an increase in α-helix from 57–62%. The corresponding estimate of β is 20%. In the helix promoting solvent, trifluoroethanol, the amount of α-helix increased to about 100%. The estimate of α-helix is slightly higher than reported [10,11]. The α-helical content is stable since dialysis of the protein in trifluoroethanol back to the original solvent gave rise to the original spectrum. In an attempt to find independent evidence to support the β estimation given in table 1, we measured the IR spectrum (fig.1). At a concentration of L7 equal to 35 mg/ml in deuterium oxide there was no evidence for any band at 1610 cm^{-1}, a frequency associated with the extended structure [12]. Thus the CD estimation of β is probably in error, and this structure is not found in this molecule.

The CD spectra of the moderate halophile are shown in fig.2. In this case, there is a well-defined

+Present address: Department of Biochemistry and Microbiology, University of Victoria, Victoria, B.C., V8W 2Y2 Canada

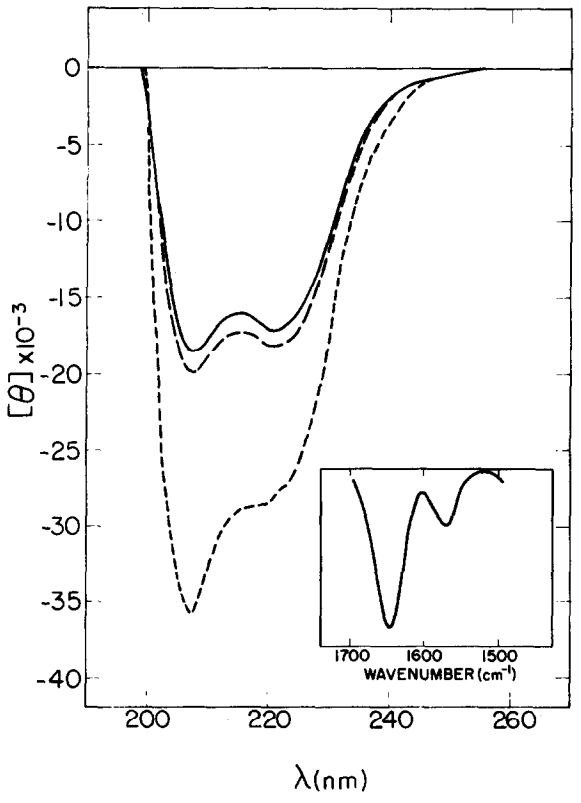

Fig.1. CD spectrum of *E. coli* L12 in 0.1 M potassium chloride, 2 mM potassium phosphate, pH 7.6 (——); in 3 M potassium chloride (– –); in 75% trifluoroethanol (– – –). Insert: infrared spectrum of EL7 in 0.1 M potassium chloride, 2 mM potassium phosphate, pH 7.6 in deuterium oxide. Concentration, 35 mg/ml; path length 0.070 mm.

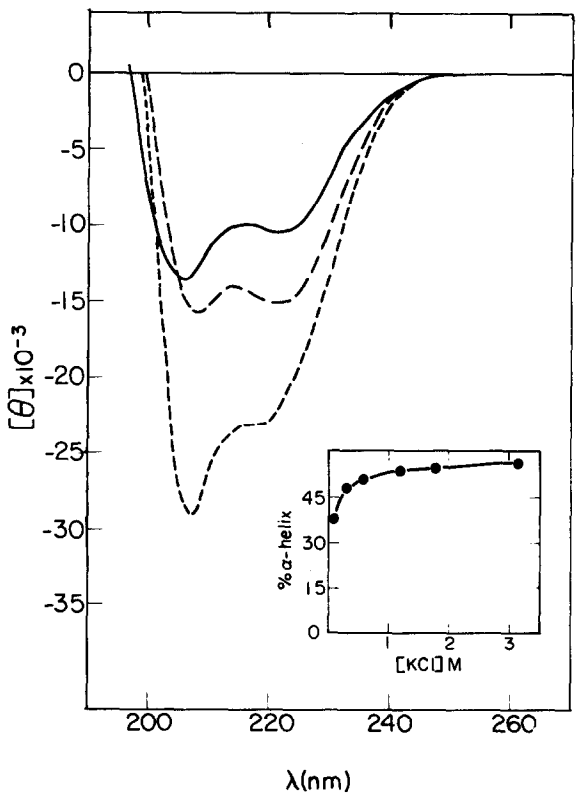

Fig.2. CD spectrum of acidic protein from moderate halophile in 0.1 M potassium chloride, 2 mM potassium, pH 7.6 (——); in 3 M potassium chloride (– –); in 75% trifluoroethanol (– – –).

Table 1
Predicted-α-helical regions by method [15] in L12 equivalent proteins

Organism	Residues in α-helix	% α-helix	
		Predicted	CD estimation
E. coli	6–14, 21–41, 43–71, 80–96, 102–120	79	67
Moderate halophile (NRCC 41227)	4–38, 46–59, 83–90, 106–119	58	56
H. cutiribrum	6–18, 34–63	55[a]	41

[a]For the first two-thirds of the protein for which the sequence has been determined

increase in the α-helicity from 35–56% as the salt concentration is increased from 0.1–3.0 M. The addition of trifluoroethanol again promotes almost total helicity in the molecule. The estimate of 56% α-helix approaches that of *E. coli*, and the results are consistent with a protein structure adapted to a somewhat higher internal salt concentration than *E. coli* [2].

Finally, *H. cutirubrum* L20 undergoes a pronounced change in its secondary structure between 0.1 M and 3 M KCl, as shown in fig.3. The secondary structure is highly dependent on the potassium chloride concentration and the protein goes through a well-defined transition centered at about 1.5 M potassium chloride corresponding to a doubling of the α-helix content (fig.3). The α-helix increases from 17–40% (fig.3), but is still substantially below that of the other proteins. It is perhaps of interest that the α-helical content of HL20 is about 50% higher than that of two other ribosomal proteins from *H. cutirubrum*, HL13 and HL19 [13], since EL12/EL7 has an unusually high α-helix content with relation to the other *E. coli* 50 S proteins [10].

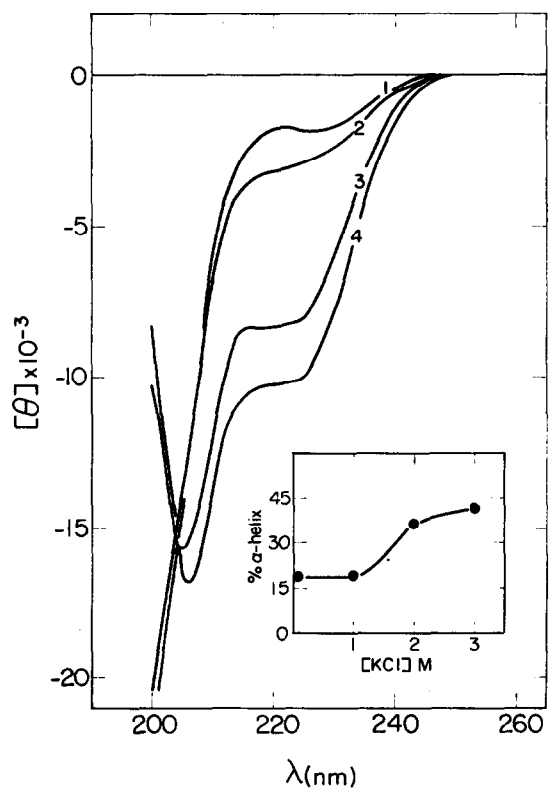

Fig.3. CD spectrum of extreme halophile, HL20, in 0.1 M potassium chloride, 2 mM potassium phosphate, pH 7.6 (1); and with 1 M (2), 2 M (3), and 4 M (4) potassium chloride.

We have examined the use of two predictive theories to attempt to correlate the sequence of L7/L12 and the moderate halophile equivalent protein with the estimated secondary structure. The method in [14] was found to be too arbitrary for these particular proteins and grossly overpredicted the amount of α-helix. The same problem occurred with the method in [15] if the prediction of most probable structure was used, since this was almost entirely α-helical. However, if the probability function for α-helix is graphed in the form of a nomogram, we find that there are regions in which residues collectively fall below the 50% level of probability (fig.4). If we exclude those below 50% we find that the predicted and measured values of α-helix agree much more closely, especially for the moderate halophile. Our prediction of 79% α-helix exceeds that of 64% in [16], but this difference does not change any of our interpretations. Their analysis of EL12 indicates even closer secondary structure homology to the equivalent moderate halophile protein than that presented here.

The nomograms of fig.4 and table 1 indicate that regions of expected α-helix are largely retained, and

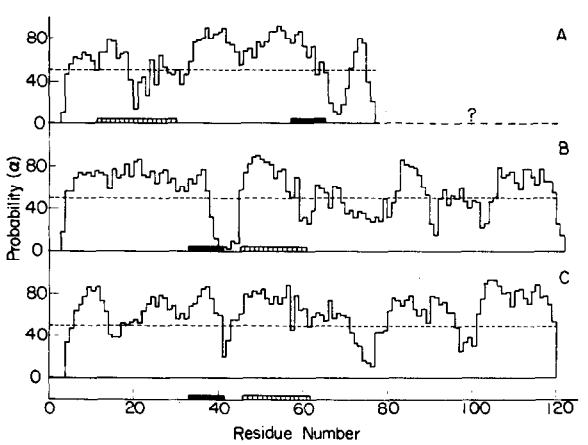

Fig.4. Nomogram of α-helix probability by method in [15] for *E. coli* L7/L12 protein (2), and the equivalent proteins from *H. cutiribrum* (A) and the moderate halophile (B). The dark and hatched blocked areas represent apparent regions of sequence homology between HL20 and the other two proteins.

suggest considerable homology in the secondary structure, for *E. coli* and the moderate halophile. There is no discernible correlation between the sequence and secondary structure homologies. The situation for the HL20 with respect to the other two is not so clear. There is much less homology between this protein and the other two. Regions of sequence homology are indicated in fig.4. Residues 12-31 in HL20 appear to have no secondary structure homology with EL12, whereas residues 57–65 do appear to have homology. At this point, the predictions indicate no well-defined secondary structure homology between HL20 and the other procaryote L12 proteins. The remaining portion of HL20 for which the sequence is not known is rich in aspartic acid, a weak α-helix former [14]. This probably accounts for the predicted helix being less than the observed from the CD spectrum (table 1).

We have also examined the molecular weights of the acidic proteins, since EL7/EL12 is known to form a stable dimer [1]. Our results indicated dimerization of EL12 with no indication of free monomer. The acidic protein from the facultative organism apparently reversibly formed a dimer under the equivalent conditions (0.1 M potassium chloride, pH 7.6). In 2 M KCl or 0.1 M HCl, HL20 aggregated to a tetramer.

We conclude that the evolutionary pressure to conserve the secondary structure in ribosomal proteins is large, and this accounts for the apparent large homology between *E. coli* and the moderate halophile. However, *H. cutirubrum* is very far removed from the other two organisms on an evolutionary scale, and the secondary structure analysis reveals no areas of well-defined equivalence with *E. coli* or the moderate halophile.

Acknowledgement

G.E.W. is a NRCC research associate.

References

[1] Möller, W., Castleman, H. and Terhorst, C. P. (1970) FEBS Lett. 8, 192–196.
[2] Matheson, A. T., Sprott, G. D., McDonald, I. J. and Tessier, H. (1976) Can. J. Microbiol. 22, 780–786.
[3] Terhorst, C., Möller, W., Laursen, R. and Wittman-Liebold, B. (1973) Eur. J. Biochem. 34, 138–152.
[4] Falkenburg, P., Yaguchi, M., Matheson, A. T., Roy, C. and Rollin, F. manuscript in preparation.
[5] Oda, G., Ström, A. R., Visentin, L. P. and Yaguchi, M. (1974) FEBS Lett. 43, 127–130.
[6] Möller, W., Groene, A., Terhorst, C. and Amons, R. (1972) Eur. J. Biochem. 25, 5–12.
[7] Ström, A. R., Oda, G., Hasnain, S., Yaguchi, M. and Visentin, L. P. (1975) Mol. Gen. Genet. 140, 15–27.
[8] Willick, G. and Zuker, M. (1978) Biophys. Chem. in press.
[9] Chen, Y., Yang, J. T. and Chan, K. H. (1974) Biochemistry 13, 3350–3359.
[10] Dzionara, M. (1970) FEBS Lett. 8, 197–200.
[11] Boublik, M., Brot, N. and Weissbach, H. (1973) Biopolymers 12, 7083–7092.
[12] Miyazawa, T. (1967) in: Poly-α-Amino Acids (Fasman, G, ed) Vol. 1, pp 69–103, M. Dekker, NY.
[13] Willick, G. E., Williams, R. E., Sendecki, W. and Matheson, A. T. (1977) submitted.
[14] Chou, P. Y. and Fasman, G. D. (1974) Biochemistry 13, 222–245.
[15] Maxfield, F. R. and Scheraga, H. A. (1976) Biochemistry 15, 5138–5153.
[16] Wittmann-Liebold, B., Robinson, S. M. L. and Dzionara, M. (1977) FEBS Lett. 81, 204–213.