INCREASED CONFORMATIONAL STABILITY OF *ESCHERICHIA COLI* ACYL CARRIER PROTEIN IN THE PRESENCE OF DIVALENT CATIONS

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1. Introduction

Acyl carrier protein (ACP) from *Escherichia coli* which functions as a coenzyme in fatty acid synthesis is an acidic protein with a pH_I of approx. 4.2 [1]. It has been shown that divalent cations at low concentrations and monovalent cations at high concentrations enhance the biological activity of the protein [2] and maintain its ordered structure when the positive charges on the amino groups are lost due to acetylation or deprotonation [3]. Because of a continued interest in the effects of divalent cations on the conformations of proteins, specifically acidic ones, and because chemical modifications of *E. coli* ACP had been evaluated by thermal denaturation measurements without considering the influence of cations [4], the effect of cations on the conformational stability of *E. coli* ACP was investigated. This report demonstrates that both monovalent and divalent cations – although the latter ones at much lower concentrations than the former ones – cause increases in the ordered structure of ACP whereas only divalent cations stabilize the conformation of ACP as evidenced by its greatly increased thermal stability.

2. Experimental procedures

ACP was isolated and purified from *E. coli* according to the procedure of Majerus et al. [5]. The protein was acetylated with [1-^{14}C]acetic anhydride as described by Majerus [6] and was found to contain an average of 4.4 mol acetate/mol ACP. This degree of acetylation indicates that the five primary amino groups of ACP had been nearly quantitatively modified. The biological activity of acetylated ACP was 75% of that of native ACP when assayed by the malonyl pantetheine–CO_2 exchange reaction [5].

Circular dichroism (CD) measurements were made on a Jasco, J-20, automatic recording spectropolarimeter with a thermostatted cell of 1 cm path-length. Stock solutions of ACP and acetylated ACP were diluted immediately before use with 10 mM Tris–HCl (pH 7.6) to give final protein concentrations of 50 μg/ml. During the thermal denaturation measurements ellipticities were recorded 5 min after a preset temperature was reached in order to assure sufficient time for establishing an equilibrium. The thermal denaturation measurements were done in duplicate or triplicate on different days with freshly prepared solutions. The accuracies of the listed transition temperatures are ±2°C.

3. Results and discussion

The CD spectrum of ACP in 10 mM Tris–HCl (pH 7.6) is shown in fig.1A, curve 3. The addition of 10 mM $CaCl_2$ to ACP gave rise to spectrum 4 in fig.1A which at 222 nm shows a 20% increase in its negative ellipticity compared with the spectrum of ACP in the absence of $CaCl_2$. This increase in the negative mean residue ellipticity corresponds according to Chen et al. [7] to an increase in helical content of ACP from 33–42%. Raising the temperature from 10°C to between 70°C and 80°C resulted in the thermal denaturation of ACP in the presence and absence of $CaCl_2$ as illustrated by spectra 2 and 1, respectively, in fig.1A. Since the spectra of thermally denatured ACP in the presence and absence of $CaCl_2$

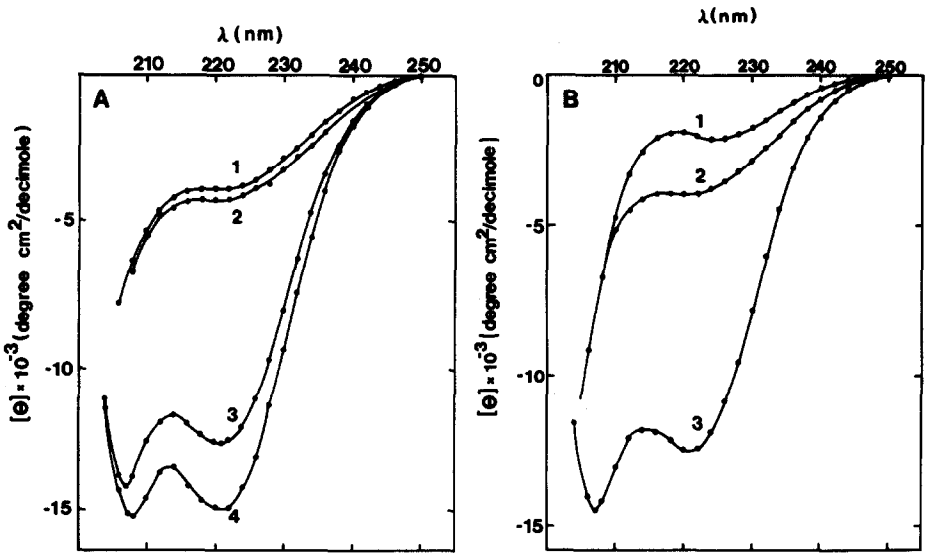

Fig.1. Circular dichroism spectra of ACP and acetylated ACP. (A) Curve 1, 70.6°C, no $CaCl_2$; Curve 2, 79°C, 10 mM $CaCl_2$; Curve 3, 10°C, no $CaCl_2$; Curve 4, 10°C, 10 mM $CaCl_2$. (B) Curve 1, 10°C, no $CaCl_2$; Curve 2, 76.5°C, 10 mM $CaCl_2$; Curve 3, 10°C, 10 mM $CaCl_2$.

are very similar, divalent cations do not appear to affect significantly the conformation of ACP at elevated temperatures. The CD spectrum of acetylated ACP measured at 10°C in Tris–HCl (pH 7.6) is shown in fig.1B, curve 1. As previously reported [3] acetylated ACP at low ionic strength and at physiological pH is devoid of any ordered structure as indicated by its small negative mean residue ellipticity at 220 nm. The addition of 10 mM $CaCl_2$ to acetylated ACP changes spectrum 1 to spectrum 3 which is nearly identical with that of highly ordered native ACP. The CD spectrum of acetylated ACP in the presence of 10 mM $CaCl_2$ at 76.5°C is shown in fig.1B, curve 2. This spectrum is indistinguishable from the spectrum of thermally denatured ACP, but is indicative of a higher degree of ordered structure than that of acetylated ACP in the absence of $CaCl_2$ (compare spectra 2 and 1 in fig.1B). The qualitative similarity of the CD spectrum of acetylated ACP (fig.1B, curve 1) with a computed CD spectrum of the random coil form which is based on five proteins of known tertiary structure [7] leads to the suggestion that acetylated ACP in the absence of salts exists in a random coil conformation while thermally denatured acetylated ACP in the presence of $CaCl_2$ as well as denatured ACP both in the presence and absence of $CaCl_2$ retain a limited degree of ordered structure.

As shown in fig.2, curve 1, the denaturation of ACP in 10 mM Tris–HCl (pH 7.6) began at 20°C and was completed around 70°C. The transition temperature (*Tr*) obtained for ACP under this condition was 43°C (see table 1), a value which is similar to the value of 46°C previously reported by Abita et al. [4]. The addition of 10 mM $CaCl_2$ resulted in a large shift of the *Tr*-value from 43–65°C (see table 1). The curve obtained for the denaturation of ACP in the presence of $CaCl_2$ (fig.2, curve 3) is indicative of a limited structural change of low cooperativity which occurred between 10°C and 50°C followed by the major unfolding event which exhibits much higher cooperativity and which began near 50°C and was completed at 80°C. A similar result was obtained when $MgCl_2$ was used instead of $CaCl_2$ (see table 1). In contrast to the pronounced stabilization of ACP towards thermal denaturation by divalent cations, KCl at a concentration of 0.1 M did not affect the transition temperature of ACP (see table 1), although it induced a significant increase in the negative ellipticity of ACP at 10°C (see fig.2, curve 2). The addition of 1 mM or 2 mM EDTA or EGTA to an ACP solution in 10 mM Tris–

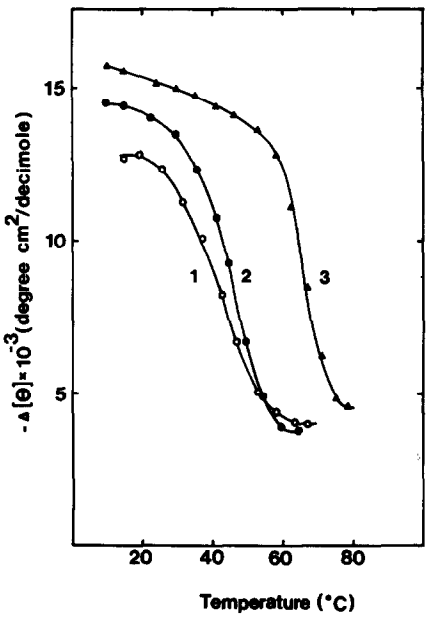

Fig.2. Thermal denaturation curves of ACP. Curve 1, no addition; Curve 2, + 0.1 M KCl; Curve 3, + 10 mM $CaCl_2$.

HCl (pH 7.6) caused a decrease in the negative ellipticity at 220 nm of approx. 5% and yielded a transition temperature of 37°C (see table 1). This finding suggests that a small amount of multivalent cations had either remained bound to ACP or had been added during the preparation of the ACP solution. A transition temperature for acetylated ACP in the absence of salts could not be determined because the protein was at 10°C devoid of any ordered structure. As shown in fig.1B, curve 3, acetylated ACP in the presence of 10 mM $CaCl_2$ had regained its ordered structure and the transition temperature under this condition was found to be 54°C (see table 1), a value

Table 1
Effect of salts on the transition temperature (*Tr*) of ACP

Sample	Addition	*Tr* (°C)
ACP	None	43
ACP	100 mM KCl	44
ACP	10 mM $CaCl_2$	65
ACP	10 mM $MgCl_2$	63
ACP	1 mM EDTA or 2 mM EDTA	37
Acetylated ACP	10 mM $CaCl_2$	54

which suggests that the acetylated ACP–Ca^{2+}-complex is more stable than native ACP but less stable than the ACP–Ca^{2+}-complex. The observation that acetylated ACP was devoid of any ordered structure at 10°C but regained it in the presence of Ca^{2+} leads to the suggestion that the degree of ordered structure and the transition temperature of 32.5°C determined by Abita et al. [4] for acetylated ACP may have been due to the presence of small amounts of divalent cations in their protein preparation.

The above data demonstrate that low concentrations of divalent cations or high concentrations of monovalent cations cause increases in the ordered structure of ACP. However, only divalent cations, which apparently bind to ACP [8], stabilize the protein towards thermal denaturation. It is interesting to note that the addition of Ca^{2+} to calsequestrin leads to changes in its secondary structure similar to those observed with ACP. Hence, the increased ordered structure and stability in the presence of multivalent cations may be a general property of acidic proteins. The findings presented here and previously [3] are important for future work aimed at studying the possible simultaneous interaction of ACP with various enzymes of the *E. coli* fatty acid synthesizing system which may exist in vivo as a multi-enzyme complex.

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