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FEBS Letters 339 (1994) 165–167

FEBS 13661

FEBS LETTERS

D-Ribose stabilizes precursor and mature ribose-binding proteins of *Escherichia coli*

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Received 15 November 1993; accepted 5 January 1994

Abstract

Heat- and guanidine hydrochloride-induced unfolding and refolding of precursor as well as mature ribose-binding proteins of *Escherichia coli* were studied in the presence of D-ribose using intrinsic tyrosine fluorescence and circular dichroism. The precursor and mature proteins have shown virtually identical unfolding-folding behavior. It was observed that D-ribose refolds partially unfolded precursor and mature ribose binding proteins into native structure and decreases the unfolding rate of the these proteins. The conformational stabilities of these proteins were found to increase with increasing D-ribose concentration.

Key words: Ribose, D-; Ribose-binding protein; Unfolding; Conformational stability

1. Introduction

The binding proteins, present in the periplasmic space of Gram-negative bacteria, are involved in the active transport and chemotaxis of various substrates. They are synthesized first as precursors in the cytoplasm, exported into the periplasm and then processed to the mature forms. It was assumed that, when a substrate is bound, the binding protein undergoes a conformational change and this protein complex, in turn, interacts with a proper membrane receptor effecting either substrate transport or chemotaxis [1–7]. This conformational change was documented for galactose-binding protein [1–5], ribose-binding protein [3], arabinose-binding protein [6], and leucine/isoleucine/valine-binding protein [7]. Various physical methods demonstrated that binding of substrates transforms the mature binding proteins into more compact forms [6,7].

It should be of interest to see whether this structural change accompanies a change in the stability of binding proteins and also whether the precursor binding proteins undergo a similar change as the mature proteins. Here, we have investigated the stability of precursor ribose-binding protein (pRBP) and mature ribose-binding protein (mRBP) of *Escherichia coli* as a part of our overall studies on the mechanism of its secretion.

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Abbreviations: RBP, ribose-binding protein; pRBP, precursor RBP; mRBP, mature RBP; GdnHCl, guanidine hydrochloride; CD, circular dichroism

2. Materials and methods

pRBP and mRBP were purified from the strains IQ87 (MC4100 secY^{ts}/pCI857, pSP107) and SP114 (NR69/pSP107), respectively, by ion exchange chromatography as described elsewhere in detail [8] but after some minor modifications. Protein concentration was determined by the Bradford method using bovine serum albumin as a standard [9]. GdnHCl was purchased from Sigma and the stock solutions were prepared daily, its concentration being determined by refractometry [10]. All experiments were done at 25°C with 2 μM protein concentration in 50 mM potassium phosphate buffer, pH 7.5. Fluorescence intensity was measured on a Jasco FP770 spectrofluorometer in a thermostated cuvette with 280 nm excitation wavelength. Thermal unfolding was monitored by following the decrease in fluorescence intensity at 303 nm (excitation at 280 nm) or the change in molar residue ellipticity at 222 nm with 1°C/min heating rate. All CD spectra were recorded on a Jasco J600 spectropolarimeter using a thermostated, 0.1 cm path length cell.

3. Results and discussion

As shown in Fig. 1A, maximum fluorescence emission for pRBP occurs at 303 nm (excitation at 280 nm) and the main effect of the denaturant was a decrease of about 45% in fluorescence intensity without any shift in the maximum wavelength. In 0.55 M GdnHCl, the fluorescence intensity was about halfway between those in 0 M and 1 M denaturant solutions showing that the proteins were partially unfolded. This result deviates somewhat from the one obtained by Teschke et al. [8] who observed about 80% unfolding at 0.55 M GdnHCl. This discrepancy may be due to the stabilizing effect of the potassium phosphate buffer used here [11]. The results with mRBP (not shown here) were essentially the same as pRBP. Analysis of the CD spectrum for the native mRBP (not shown here) using the reference spectra obtained from

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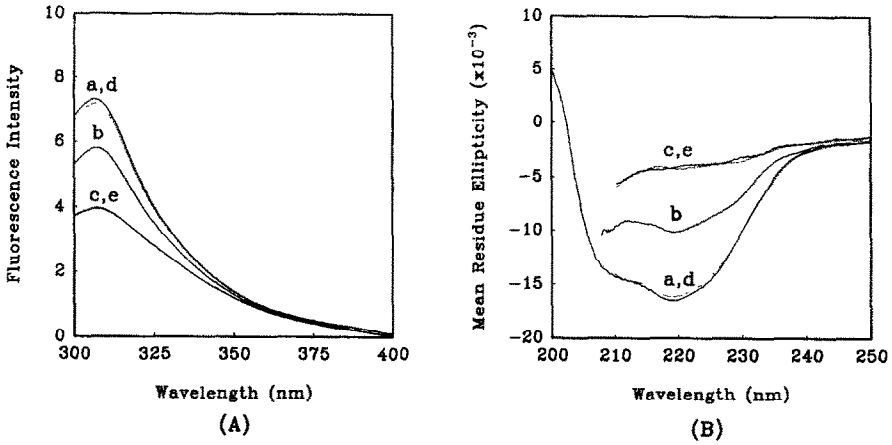

Fig. 1. Recovery of native structure from partially unfolded pRBP by D-ribose as observed by the fluorescence (A) and CD (B) spectra. pRBP (2 μM) was initially incubated in 0 M (a), 0.55 M (b) and 1 M GdnHCl (c) solutions containing 50 mM potassium phosphate (pH 7.5) at 25°C for 24 h. The spectra traced with dotted lines were obtained after adding 100 μM D-ribose to the protein solutions in 0.55 M (d) and 1 M GdnHCl (e), respectively, and then incubating for additional 24 h.

five proteins (myoglobin, lysozyme, ribonuclease A, papain, and lactate dehydrogenase) [12] gave 44% α-helix, 20% β-sheet, 12% β-turns, and 24% random structure. These agree well with the X-ray crystallographic data (45% α-helix, 23% β-sheet, 10% β-turns, and 22% random structure) [13]. The native pRBP, also determined by CD as shown in Fig. 1B, had virtually the same secondary structures (44% α-helix, 20% β-sheet, 11% β-turns, and 25% random structure). Fig. 1 shows that D-ribose folds partially unfolded pRBP and mRBP (not shown) into the native structures but D-ribose had no effect on fully unfolded proteins. We also observed that D-ribose decreases the unfolding rates of the pRBP and mRBP with increasing concentration of D-ribose and in the presence of 1 mM D-ribose, pRBP and mRBP could not be unfolded by 1 M GdnHCl (Fig. 2). We did not observe any effect of D-ribose on the refolding kinetics.

Fig. 3 (solid lines) shows the tyrosine fluorescence intensity of both pRBP and mRBP at 303 nm as a function of temperature. The initial slow decrease is conformation-independent but the subsequent sharp decrease is due to denaturation. Both protein solutions showed identical conformational change with a transition temperature near 55–56°C, suggesting that thermal stabilities of these two proteins are similar. Addition of D-ribose resulted in an increase in the transition temperature. Fig. 3 (dotted lines) also presents the thermal denaturation of RBPs in the absence and presence of

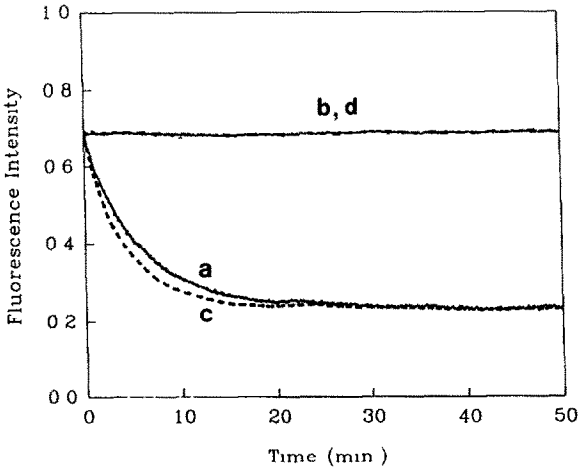

Fig. 2. Kinetic progress curves of unfolding of pRBP (a,b) and mRBP (c,d) measured by the fluorescence changes at 303 nm (at 25°C) in the absence (a,c) and presence (b,d) of 1 mM D-ribose. The unfolding was initiated by a 1/20 dilution of the native protein solutions with 1 M GdnHCl unfolding buffer (50 mM potassium phosphate, pH 7.5). Final protein and GdnHCl concentrations were 2 μM and 0.05 M, respectively.

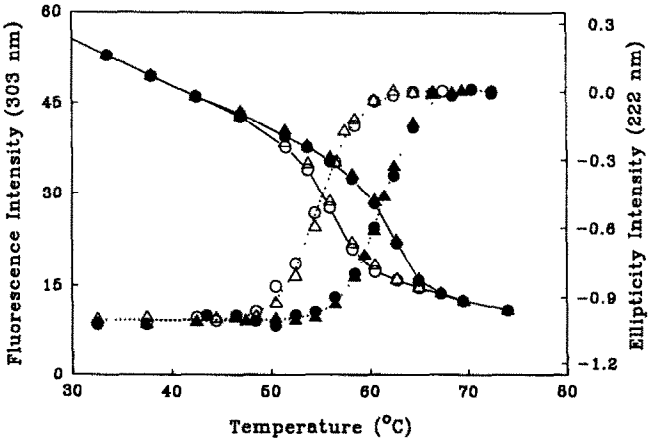

Fig. 3. Temperature dependence of pRBP (circle) and mRBP (triangle) fluorescence (solid lines) and relative ellipticity (dotted lines) in the absence (open) and presence of 50 μM D-ribose (closed). All solutions contained 50 mM potassium phosphate (pH 7.5) and 2 μM protein. Fluorescence intensity at 303 nm was measured with 280 nm excitation wavelength. The relative ellipticity was measured at 222 nm.

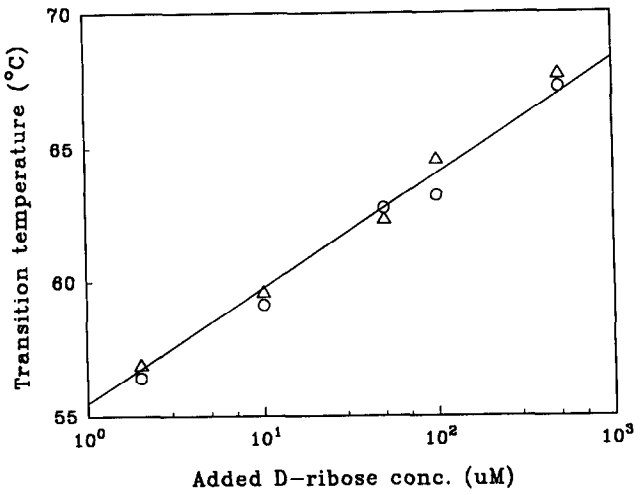

Fig. 4. Concentration dependent increase of transition temperature midpoints measured by the fluorescence intensity at 303 nm.

D-ribose monitored by CD which gives essentially the same transition temperature as obtained by fluorescence. Fig. 4 presents the increase in the transition temperature with increasing D-ribose concentration. These results show that D-ribose increases the conformational stability of both pRBP and mRBP to the same extent. The effect of D-ribose concentration on the transition temperatures as determined by CD gave the same results (data not shown). D-Allose, D-xylose, D-arabinose, and D-galactose at 100 μM concentrations had no effect on the transition temperature of the RBPs. It has been known that D-allose binds to mature RBP albeit with a greatly reduced affinity (dissociation constants : 3×10^{-4} μM for allose as opposed to 3×10^{-7} μM for ribose) [14]. D-allose, however, had no discernible effect on the transition temperature of pRBP and mRBP.

This study unequivocally demonstrated an increase in the stability of both pRBP and mRBP by D-ribose which apparently coincides with the conversion into a more compact structure for the case of mRBP. There is no comparative study on the structural change for the precursor protein. Although we do not know why this type of structural change results in the stabilization, there are a number of reports on this phenomenon [15–19].

It is of interest that the stability increase by D-ribose occurs to both pRBP and mRBP to an equal extent, indicating structural similarity between these two proteins despite of the existence of N-terminal extension of signal peptide in the precursor protein. The secondary structure determined by CD and equilibrium unfolding-folding behavior of pRBP (Fig. 1) and mRBP are essentially the same supporting the notion that these have basically the same structure.

The translocation competent pRBP has a somewhat loose structure and the effect of substrate-induced stabilization on the translocation of this protein is an interesting question. The possibility of the ligand-induced stabilization being involved in the regulation of secretion itself of pRBP may be ruled out from our observation that D-ribose had no effect on the in vivo translocation of pRBP (data not shown).

Acknowledgements: We thank Professor Thong-Sung Ko of the Department of Biochemistry, Chungnam National University for kindly allowing us an extensive use of spectropolarimeter.

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