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Commentary

A comment on: 'The aromatic amino acid content of the bacterial chaperone protein groEL (cpn60): Evidence for the presence of a single tryptophan', by N.C. Price, S.M. Kelly, S. Wood and A. auf der Mauer (1991) FEBS Lett. 292, 9–12

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1. INTRODUCTION

The groEL protein of *Escherichia coli*, a tetradecamer of ~60 kDa subunits, functions as an ATP-dependent molecular chaperone in protein folding. Price et al. recently published a spectroscopic analysis of purified groEL in which they reported the presence of a single tryptophan per groEL subunit. The presumed absence of tryptophan from groEL, indicated by the DNA-derived sequence [1], had formed the basis for the conformational analysis of groEL-bound substrate proteins via their intrinsic tryptophan fluorescence [2].

We have re-analyzed the spectroscopic properties of our groEL preparations and conclude that groEL protein does not contain tryptophan. This is based on the following observations.

2. RESULTS

groEL protein was purified from *E. coli* cells bearing the plasmid pOF39 [3] by a modification of published procedures [2,3]. Cells were grown at 37°C. Based on Coomassie staining groEL was about 97% pure. Protein contents were determined by quantitative amino acid analysis which gave composition data within 5–10% accuracy and indicated the presence of 6–8 Tyr per groEL subunit. The absorption at 280 nm for 0.1% solutions (A_{280}) of groEL in 6 M guanidinium-Cl was in the range of 0.20 to 0.23 (Table I). Based on the presence of 7 Tyr in groEL [1] and on the molar absorbance of Tyr and Trp, this suggested the presence of contaminating protein. The amounts of Trp (0.23–0.44 per groEL subunit) in the groEL fractions were determined by measuring the fluorescence of the guanidinium-Cl denatured protein [4]. The Trp content of two control proteins, bovine α-lactalbumin (4 Trp) and β-lactamase of *Staph. aureus* (no Trp), was determined correctly (Table I).

Silver staining demonstrated the presence of protein contaminants in our groEL preparations. A groEL fraction thought to be essentially pure by Coomassie staining contained a multitude of polypeptide bands in the molecular mass range of 14–150 kDa (Fig. 1). These polypeptides cofractionated with groEL through all of the purification steps, apparently in association with the chaperone. Incubation with Mg-ATP released a large part of this protein from the chaperone resulting in a 50% reduction in the Trp content of the re-isolated groEL (Table I, Fig. 2). It is known that groEL-bound substrate proteins are highly sensitive to protease [2]. When groEL was incubated at 0°C with a low concentration of proteinase K that leaves the chaperone complex intact, the number of contaminating protein bands visible on silver stained gels decreased, as did the Trp content of the groEL fraction (Table I).

3. CONCLUSIONS

Our results are in disagreement with the finding of a single Trp per groEL subunit. The absence of Trp in the published sequence of groEL [1] is further supported by three additional lines of evidence: (i) The low A_{280} of groEL fractions. (ii) The fluorescence measurements

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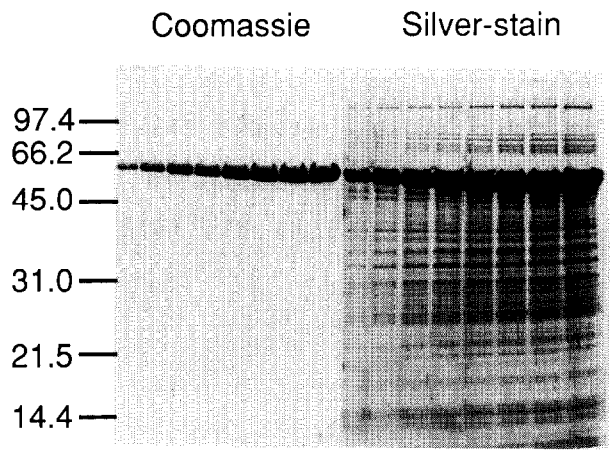

Fig. 1. SDS-PAGE of purified groEL. 2.5, 5.0, 7.5, 10.0, 12.5, 15, 17.5 and 20 μg of groEL #2 were analyzed twice. One half of the gel was stained with Coomassie and the other was silver stained. The positions of molecular weight markers are indicated in kDa.

Table I

$A_{280.1\ cm}$ for 0.1% protein and Trp content of protein samples

Protein	A_{280}	Trp/Protein
groEL #1	0.214	0.44
groEL #1 + MgATP	n.d.	0.21
groEL #2	0.199	0.27
groEL #2 + PK	0.190	0.10
groEL #3	0.207	0.23
groEL #4	0.225	0.26
α-Lactalbumin	2.008	3.79
β-Lactamase	0.579	0.12

groEL #1–5 are different preparations. groEL #1 was analyzed before and after 30 min incubation with 1 mM ATP/5 mM MgAc at 25°C; groEL #2 before and after 12 min incubation with 1 μM proteinase K (PK) at 0°C followed by re-isolation of groEL by gel chromatography. Trp content was determined via Trp fluorescence [4] and is given in mol per mol of protein or groEL monomer.

yielding values as low as 0.1 Trp per groEL monomer. (iii) The reduction of the Trp content by incubation with Mg-ATP or by mild proteolytic treatment of groEL.

The published A_{280} values of purified groEL vary considerably. Fo example, Price et al. recommended a value of 0.285 while Fisher recently used a value of 0.21 corresponding to 0.2–0.3 Trp per groEL monomer [5]. The variation in A_{280} is apparently due to contamination by Trp-containing proteins and peptides. groEL preparations contain a multitude of bound polypeptides of a wide size range that can easily escape detection by Coomassie staining, the technique used by Price et al. to estimate the high purity of their groEL. We assume that groEL interacts with many *E. coli* polypeptides during de novo folding and under cellular stress. Notably, the amount of groEL-bound contaminants increases when treatment of the cells at 42°C is used to enhance groEL expression.

Price et al. used a plasmid, pND5, for overexpression of groEL that is different from the one employed by us. Due to a mutation, a Trp-containing protein may in fact have been produced. We feel, however, that the presence of groEL-bound contaminants deserves consideration as an alternative explanation for the results of these authors. An analysis of their groEL by proteolytic cleavage and sequencing would be helpful in clarifying the situation.

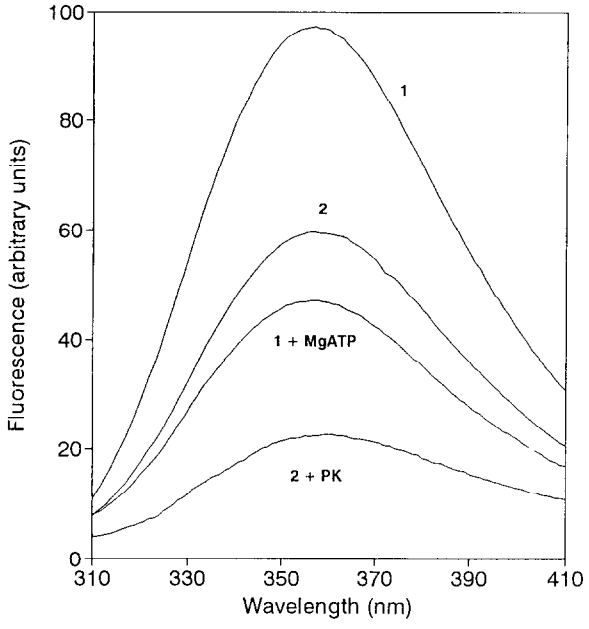

Fig. 2. Trp fluorescence of groEL excited at 295 nm. Emission spectra of 10 μM groEL (monomer) in 6 M guanidinium-Cl. (1) groEL #1 before and after treatment with Mg-ATP. (2) groEL #2 before and after treatment with PK (see Table I).

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