X-RAY DETERMINATION OF THE GDP-BINDING SITE OF *ESCHERICHIA COLI* ELONGATION FACTOR Tu BY SUBSTITUTION WITH ppGpp

Dietrich SUCK and Wolfgang KABSCH+

European Molecular Biology Laboratory, Postfach 10.2209, 6900 Heidelberg and *+Max-Planck-Institut für medizinische Forschung, Abteilung Biophysik, Jahnstr. 29, 6900 Heidelberg, FRG*

Received 23 February 1981

1. Introduction

Amino acid starvation or lack of any essential amino acid leads to the so-called stringent response in bacterial cells (reviews [1–3]). Characteristic of the stringent response is the immediate cessation of most RNA synthesis. The stringent factor, a protein which is a product of the *relA* gene, is the enzyme catalyzing the transfer of a pyrophosphate group from ATP to the 3-OH groups of either GDP or GTP giving rise to the synthesis of ppGpp or pppGpp, respectively [4]. These unusual guanosine nucleotides (originally termed magic spots I and II [5]) are accumulated, probably as a result of binding of uncharged tRNAs in the ribosomal acceptor site. ppGpp then inhibits transcription by interferring with the RNA-polymerase: DNA interaction [6,7].

In [8] the interaction of ppGpp with *E. coli* polypeptide elongation factors Tu (EF-Tu) and G (EF-G) was studied. The exchange reaction of [^{3}H]GDP in the EF-Tu · GDP complex was found to be inhibited in a competitive manner by ppGpp and that ppGpp has a markedly stabilizing effect on free Tu. The conclusion from these results was that ppGpp forms a stable complex with Tu, probably interacting at the same site as GDP or GTP. The affinity of the Tu · ppGpp complex was estimated to be one order of magnitude lower than that of the Tu · GDP complex [8]. Similar results were obtained [9] were a K_d of 8×10^{-9} M was determined.

In [10] we described the crystal structure analysis of trypsin-modified EF-Tu · GDP complex at 6 Å resolution. Using difference Fourier techniques we have been able to locate the GDP-binding site by soaking crystals of this complex with ppGpp. GDP and ppGpp were found to bind at the same site.

2. Materials and methods

ppGpp was generously provided by Dr D. Richter, University of Hamburg. Orthorhombic crystals of trypsin-treated EF-Tu [10] were soaked for 7 days in a solution containing 2×10^{-3} M ppGpp and 11.5% polyethyleneglycol in 50 mM Tris–HCl buffer (pH 7.0).

Three-dimensional X-ray reflection intensities to 6 Å resolution were measured on a Syntex $P2_1$ automatic 4-circle diffractometer using Ni-filtered CuKα radiation. The scan width used in the ω-scan mode was 0.65°, the scan rate was 0.7°/min; 5 standard reflections were monitored every 200 reflections and used to correct for radiation damage; 2256 intensities to 6 Å resolution were collected from one crystal and corrected for absorption and polarization effects. For further details of the data collection see [10]. Scaling of the amplitudes to the native data yielded an R_c-factor of 13.5%. A difference Fourier map was calculated using the 6 Å multiple isomorphous replacement phases.

3. Results and discussion

The difference Fourier map contained two prominent positive peaks of equal height which exceeded all other peaks in the map by a factor of $\geqslant 3.5$. The fractional coordinates of the 2 prominent peaks, as obtained from centric parameter refinement are:

$x_1 = 0.8080 \quad y_1 = 0.1109 \quad z_1 = 0.4471$

$x_2 = 0.7754 \quad y_2 = 0.1366 \quad z_2 = 0.6467$

Elsevier/North-Holland Biomedical Press

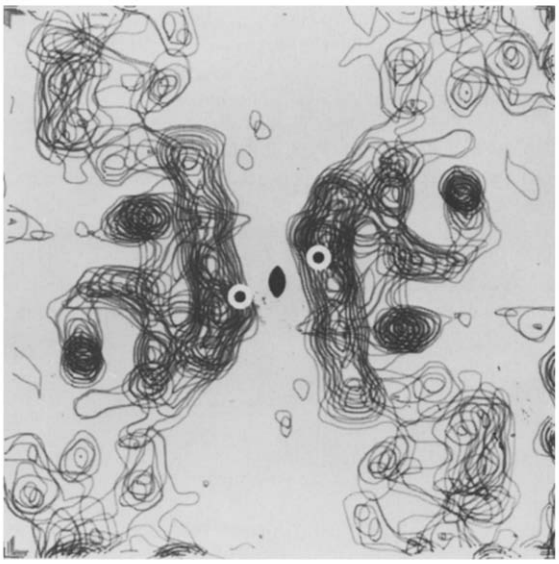

Fig.1. Part of the averaged 6 Å electron density map of the EF-Tu · GDP complex showing the ppGpp positions (◉) in the 2 protein molecules in the asymmetric unit. Six contiguous sections perpendicular to the non-crystallographic 2-fold axis (indicated in the centre of the map) are shown with contour lines drawn at 10, 20, ... 90% of the maximum density. The distance between sections is 2 Å; the size of the map is 80 Å × 80 Å.

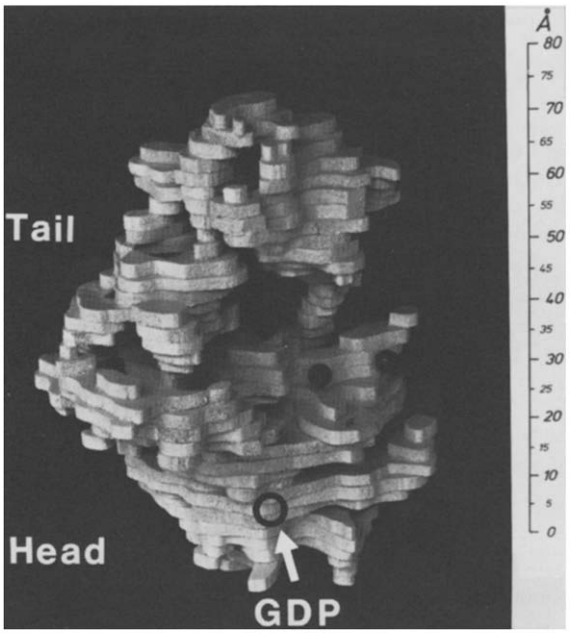

Fig.2. GDP-binding site of *Escherichia coli* EF-Tu. A balsa wood model of the EF-Tu · GDP complex derived from the averaged 6 Å electron density map is shown. The GDP- and ppGpp-binding site at the surface of the head domain is indicated.

Both positions are connected by the same non-crystallographic 2-fold axis relating the two EF-Tu molecules in the asymmetric unit of the $P2_12_12_1$ crystals [10] showing that there is 1 ppGpp bound/protein molecule at an equivalent site (fig.1). This site is only 6 Å away from the point of highest density in the 6 Å map at the end of a density rod most probably representing a 20 Å long α-helix. As shown in fig.2, this binding site is located at the surface of the tight or head domain [10] of the EF-Tu molecule.

The difference Fourier contains no significant positive or negative density in the vicinity of the 2 prominent peaks. This proves that ppGpp has in fact substituted GDP with the 5′-pyrophosphate groups of GDP and ppGpp occupying identical positions. The 2 peaks are due to the additional 3′-pyrophosphate groups of the ppGpp molecules. If ppGpp were bound at a different site, one would expect 2 positive peaks separated by ~7 Å for the 5′- and 3′-pyrophosphates or at least a smeared out positive density region. Thus, the biochemical evidence, suggesting that GDP and ppGpp bind at the same site [8,9], is in agreement with the results of this crystallographic study.

None of the mercury sites of the methylmercury-acetate derivative used in the 6 Å structure analysis [10] is closer than 20 Å to the ppGpp or GDP-binding site. Assuming, that the sulfhydryl groups of EF-Tu are labelled by the mercury derivative, this would mean, that none of the cysteines is in the vicinity of the bound GDP. In agreement with this result it was found that a sulfhydryl group is not essential for the binding of GDP to EF-Tu from *Bacillus stearothermophilus* [11].

As far as can be judged from the data in [12], the binding site proposed for GDP is the same as that found by us through substitution with ppGpp.

Acknowledgements

We thank Drs R. Leberman and F. Wittinghofer for critically reading the manuscript.

References

[1] Cashel, M. (1975) Annu. Rev. Microbiol. 29, 301–318.
[2] Gallant, J. (1979) Annu. Rev. Genet. 13, 393–415.
[3] Gallant, J. and Lazzarini, R. A. (1975) in: Protein Synthesis: A Series of Advances (McConkey, E. H. ed) Dekker, New York.
[4] Cashel, M. and Kalbacher, B. (1970) J. Biol. Chem. 245, 2309–2318.
[5] Cashel, M. and Gallant, J. (1969) Nature 221, 838–841.
[6] Haseltine, W. A., Block, R., Gilbert, W. and Weber, K. (1972) Nature 238, 381–384.
[7] Haseltine, W. A. and Block, R. (1973) Proc. Natl. Acad. Sci. USA 70, 1564–1568.
[8] Arai, K., Arai, N., Kawakita, M. and Kaziro, Y. (1972) Biochem. Biophys. Res. Commun. 48, 190–196.
[9] Miller, D. L., Cashel, M. and Weissbach, H. (1973) Arch. Biochem. Biophys. 154, 675–682.
[10] Kabsch, W., Gast, W. H., Schulz, G. E. and Leberman, R. (1977) J. Mol. Biol. 117, 999–1012.
[11] Wittinghofer, A. and Leberman, R. (1979) FEBS Lett. 101, 153–156.
[12] Morikawa, K., LaCour, T. F. M., Nyborg, J., Rasmussen, K. M., Miller, D. L. and Clark, B. F. C. (1978) J. Mol. Biol. 125, 325–338.