Proteins from the prokaryotic nucleoid

The interaction between protein NS and DNA involves the oligomeric form of the protein and at least one Arg residue

Matilde Lammi, Maurizio Paci† and Claudio O. Gualerzi+

Max-Planck-Institut für Molekulare Genetik (Abteilung Wittmann), Berlin, Germany and *Department of Cell Biology, University of Calabria, Arcavacata di Rende (CS), Italy*

Received 2 February 1984; revised version received 14 March 1984

The interaction of the prokaryotic DNA-scaffolding proteins NS (from *Escherichia coli*) and BS-NS (from *Bacillus stearothermophilus*) with DNA has been investigated. Upon binding of NS to DNA, the resolution of its 400 MHz ^{1}H-NMR spectrum is lost, due to the broadening of all resonance lines. These effects are reversed by increasing the ionic strength. Since with increasing amounts of DNA added all resonances broaden progressively and simultaneously without prior selective loss of the spectral features due to the tertiary and quaternary structure of the protein, it is suggested that NS binds to DNA in the aggregated form (octameric?) and without gross alteration of its tertiary structure. By selective chemical modification of BS-NS it was found that at least one Arg residue, located in the major hydrophilic, positively charged, conserved peptide of the protein (positions 51–70) is necessary for the interaction of BS-NS with DNA.

Bacterial chromatin *DNA-scaffolding* *Protein* *DNA binding* *Chemical modification* *NMR spectroscopy*

1. INTRODUCTION

In recent years, several DNA-scaffolding proteins (histone-like proteins) have been isolated from various eubacteria and archaebacteria. In *Escherichia coli*, the most abundant of these proteins are NS* (NS1 + NS2) and H-NS [1–5]. NS1 and NS2 are two polypeptides (approx. 9 kDa each) displaying close to 70% sequence homology [6]. H-NS is a larger protein (≃16 kDa) apparently unrelated to NS, at least as far as primary structure is concerned [5]. Unlike the case of *E. coli*, other bacteria so far investigated (e.g., *Bacillus stearothermophilus*) contain only one type of the lower molecular mass protein, homologous to both NS1 and NS2 [7].

The ^{1}H-NMR spectra of NS1, NS2 and NS are characterized by the presence of a large number of high-field perturbed resonances of stacked Phe residues, several shielded and deshielded methyl resonances and backbone NH protons quite inaccessible to the solvent [8]. These features arise primarily from strong hydrophobic quaternary interactions [8]. Crosslinking experiments have indicated that the aggregated state of NS is the tetramer or, possibly, the octamer ([5] and Losso et al., in preparation) with the basic unit consisting of heterotypic dimers [8,9]. From these and related studies (e.g., X-ray crystallography [10]) the elucidation of the structural organization of these

+ To whom correspondence should be addressed
† Permanent address: Dept. of Chemistry, University of Rome 'Tor Vergata', Rome I-00100, Italy

* In the absence of a uniform nomenclature, we refer to the two basic low-M_r DNA-binding proteins of *E. coli* as NS1 and NS2 since as in [3] we prepare these proteins from a high-salt ribosomal wash and since this is the name first given to the two separated proteins [3] (earlier thought to be a single polypeptide). The native complex of NS1 and NS2 is referred to as NS

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

proteins is gradually emerging. By contrast, very little is known concerning the mode and the molecular basis for the interaction between these proteins and DNA.

We have here investigated the interaction of DNA with NS from *E. coli* and *B. stearothermophilus* (BS-NS) by means of ^{1}H-NMR spectroscopy using deuterated DNA and by Millipore filtration using radioactive DNA and protein subjected to selective chemical modifications. We show that NS interacts with DNA in its aggregated form, that the interaction involves at least one Arg residue and we identify a hydrophilic, positively charged conserved region of NS responsible for the interaction with DNA.

2. MATERIALS AND METHODS

E. coli NS and *B. stearothermophilus* BS-NS were purified to electrophoretic homogeneity as in [11]. ^{14}C-labelled DNA (619 cpm/μg) and 84% deuterated, fragmented (average size 10 kb) *E. coli* DNA were kindly provided by Drs C.L. Pon and A. Miano. ^{1}H-NMR spectra were obtained as described in the legend to fig.1. Arginine modification with 2,3-butanedione (Serva) [12] and rose bengal-photosensitized oxidation of histidines were carried out as in [13]. Analytical protein chemical techniques were performed as in [14].

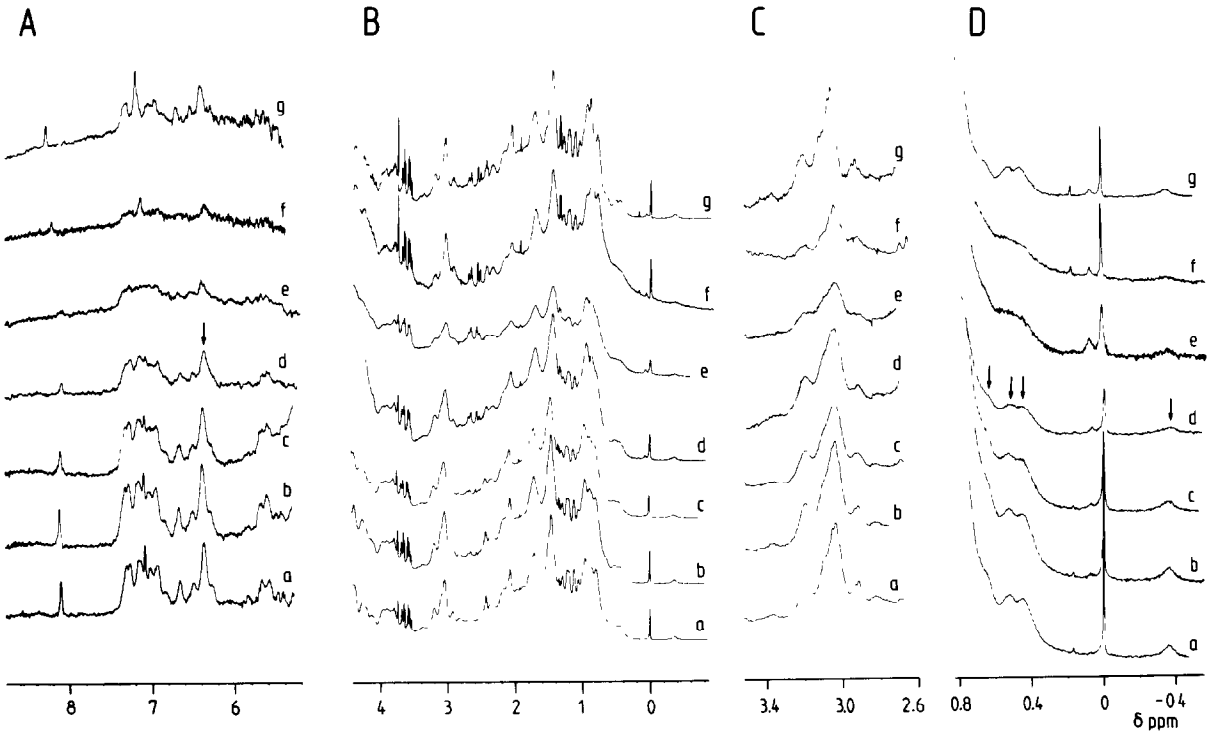

Fig.1. Effect of DNA binding on the ^{1}H-NMR spectrum of NS. ^{1}H-NMR spectra were obtained in standard 5-mm tubes at 19 ± 1°C on 5 mg/ml protein samples (≃5.5 × 10^{-4} M) in 10 mM K–phosphate buffer (pH 7.5) (meter reading) containing the indicated amounts of deuterated, fragmented DNA and, unless otherwise specified, 100 mM KCl (suprapur, Merck) in 99% D_2O (Merck). The spectra were recorded after 1000–4000 transients on a Bruker WM-400 spectrometer operating at 400 MHz with 4 μs (~30°) pulse, 6000 Hz spectral width and relaxation delay of 2.0 s. Magnetization decays obtained in the quadrature detection mode were accumulated on 16 K of memory. Sensitivity enhancement by 0.4 Hz exponential multiplication was applied. HDO was partially suppressed using an inverse gated pulse irradiation technique for a duration of 1.5 s. Spectra: (a) NS alone; (b) NS:DNA = 40:1 (w/w); (c) NS:DNA = 20:1 (w/w); (d) NS:DNA = 7:1 (w/w); (e) NS:DNA = 3.5:1 (w/w); (f) same as in e at 0.4 M KCl; (g) same as e at 1.0 M KCl. A, aromatic region; B, aliphatic region; C and D are enlarged portions of B. Arrows: (in A) a perturbed Phe resonance characteristic of NS1-NS2 heterotypic aggregates [8]; (in B) ring current shifted methyl resonances due to NS tertiary and quaternary structure [8].

3. RESULTS AND DISCUSSION

In the first experiment, increasing amounts of deuterated fragmented DNA were added to a fixed amount of NS (fig.1, spectra a–e). As seen from the figure, this causes the progressive broadening of all the resonances, without any specific modification of the spectrum, except for a slight upfield shift of the δCH_2 Arg band at 3.2 ppm [8]. These effects are not (or only minimally) due to the increase in viscosity in the system brought about by the addition of DNA, since the resonances due to preparation impurities remain relatively sharp and the broadening effect can be reversed by increasing the ionic strength (fig.1, spectra f,g). Thus, the disappearance of the protein spectrum indicates that an exchange dynamics exists between free and DNA-bound proteins; the increase in the correlation time of the bound species causes an increase in the relaxation rate which, probably together with a medium-low rate of exchange, brings about the broadening of the resonances of NS until they virtually disappear. The increase in the K_d of the DNA–NS complex caused by the addition of KCl results in an increase in the concentration of the free species in solution and the consequent reappearance of the resonance bands characteristic of NS.

It can also be seen from the figure that the ring current shifted methyl resonances (fig.1D) due to the tertiary and quaternary structure of NS [8] as well as the pattern of Phe resonances perturbed upfield (fig.1A) because of Phe ring stacking in the quaternary structure of NS [8] broaden and disappear simultaneously or even somewhat later than the other resonances. This indicates that the interaction of NS with DNA does not result in a gross alteration of the tertiary structure of the protein nor of its aggregation equilibrium (i.e., dissociation of NS aggregates is not a prerequisite for the binding). As shown above, the spectrum obtained in the presence of DNA at a high (e.g., 1 M) KCl concentration is overall very similar to that of the free protein (cf. spectra g and a of fig.1). Noteworthy, however, is the incomplete reappearance of the δCH_2 band of Arg at 3.2 ppm; this behaviour which is best seen at a higher DNA:protein ratio is particularly evident when compared to that of the nearby ϵCH_2 band of lysine (fig.2). Since no such effect was found upon

Fig.2. Involvement of δCH_2Arg in NS binding to DNA. Spectral conditions were as described under fig.1. Upper spectrum: a 1:1 (w/w) mixture of NS and deuterated, fragmented DNA at 100 mM KCl; lower spectrum: the same sample containing 2 M KCl. Only the δCH_2Arg and ϵCH_2Lys bands are shown. The partial reappearance of the δCH_2Arg resonances is indicated by the arrow. Each division on the abscissa corresponds to 0.1 ppm.

increase of the KCl concentration in the absence of DNA [8], this must be attributed directly or indirectly to the presence of the nucleic acid. This effect is probably due to a residual immobilization of the Arg residues in spite of the high ionic strength and suggests an involvement of Arg residues in the interaction between NS and DNA.

Direct evidence for this premise was sought by selective chemical modification of Arg residues with 2,3-butanedione. For this purpose, we chose to work with the NS protein extracted from *B. stearothermophilus* (BS-NS), since the presence of only one type of subunit in this protein should simplify the protein-chemical identification of the reacted residues. Thus, samples of BS-NS were reacted with 2,3-butanedione for various lengths of time; after dialysis against borate-containing buffer, increasing amounts of the modified proteins were tested for their capacity to retain radioactive DNA on nitrocellulose filters and the extent of Arg modification of each sample was determined by amino acid analysis. As seen from fig.3, the DNA-binding capacity of BS-NS is rapidly lost (80% after 10 min) following incubation with 2,3-butanedione. The loss of activity, however, does not parallel the loss of arginines; the

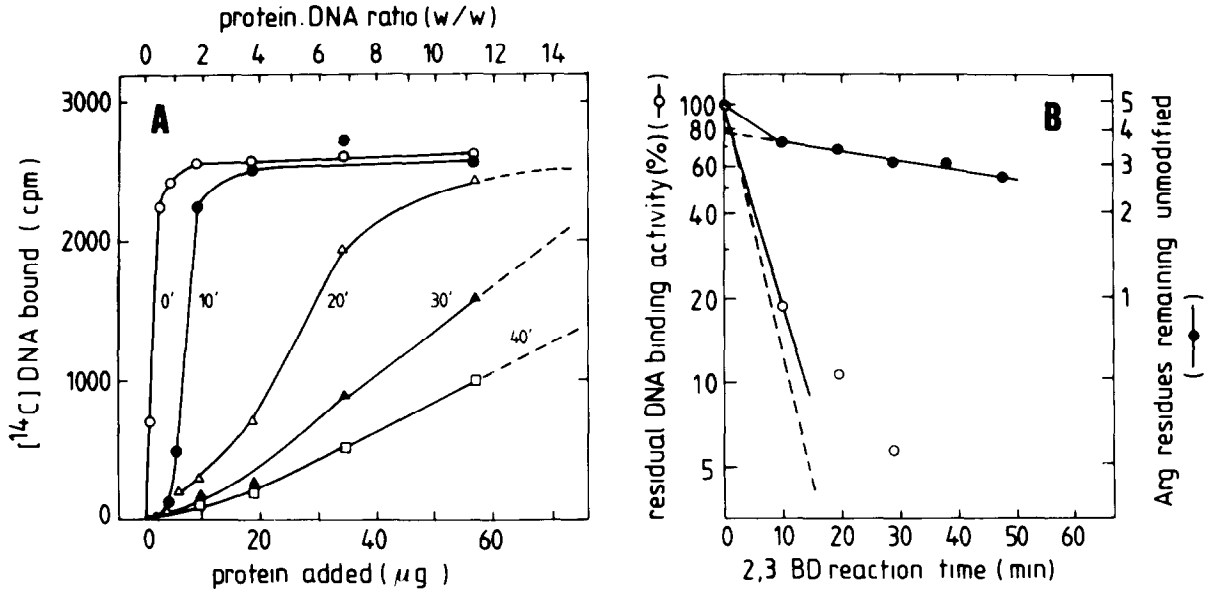

Fig.3. Inactivation of the BS-NS binding capacity following reaction with 2,3-butanedione. A, DNA binding activity of 2,3-butanedione – modified BS-NS; B, kinetics of activity and arginine loss in BS-NS. BS-NS (4.6 mg) was incubated at 25°C in 6.0 ml of 100 mM Hepes buffer (pH 8.0) containing 50 mM Na borate and 10 mM 2,3-butanedione (Serva). At the indicated times 1.0 ml aliquots were withdrawn and the reaction stopped by addition of 100 μl of arginine (24 mg/ml, pH 7.9). The protein samples to be used for amino acid analysis were exhaustively dialysed against 2% acetic acid, lyophilized and subjected to acid hydrolysis [14]. The samples to be assayed for their DNA binding capacity were exhaustively dialysed against 20 mM Tris–HCl (pH 7.2) containing 200 mM NH_4Cl, 50 mM Na borate; 1 mM NaEDTA, 10% glycerol. For the DNA binding reactions 240 μl of 0.1 × SSC containing 50 mM Na borate (pH 7.5) and 5 μg [^{14}C]DNA (619 cpm/μg) were mixed with the indicated amounts of modified BS-NS in 60 μl of the above dialysis buffer. After incubation for 15 min at 37°C, the DNA–protein complexes were collected onto Millipore filters (0.45 μm) which had been presoaked for 1 h at 22°C in 20 mM Tris–HCl (pH 7.2), 100 mM KCl, 1 mM NaEDTA, 5 mM $MgCl_2$, 5 mM βMetOH, 50 μg/ml BSA and rinsed with the above buffer without BSA. The complexes on the filters were washed with three 3-ml portions of 0.1 × SSC containing 20 mM Na borate and quantified by liquid scintillation counting. The calculated rate at which the fast reacting Arg residue is modified is shown by the dashed line.

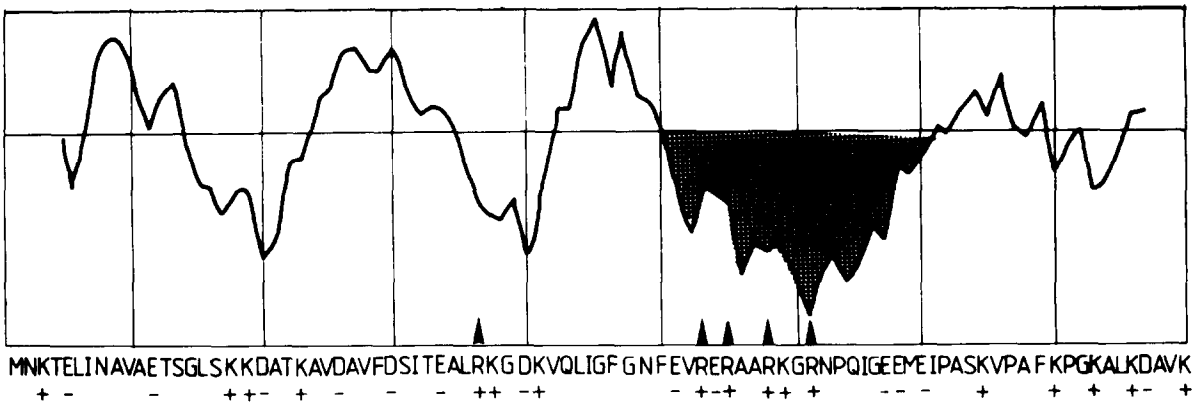

Fig.4. Hydropathic character of BS-NS. The primary structure of BS-NS [7] was analyzed according to the computer program in [15] to display the hydrophobic (above the horizontal line) and the hydrophilic (below the horizontal line) regions of the protein. The arrows indicate the positions of the 5 Arg residues in the primary structure of BS-NS. The sign (+ or −) indicates the charge of the amino acid.

inactivation is rapid and is accompanied by the loss of approx. 30% of the residues (i.e., 1.5/5), while the modification of the remaining residues follows at a slower rate. Comparison of the apparent first order rate of BS-NS inactivation with that calculated for the modification of a single fast-reacting arginine, suggests that it is the modification of this residue which causes the loss of the DNA-binding capacity of the protein (fig.3B).

Hydrophobicity analysis of the BS-NS primary structure according to the computer program in [15] reveals the existence of 3 regions (6–14; 25–34; 43–50) with strong hydrophobic character, and 3 regions (15–24; 35–42; 51–70) with strong hydrophilic character (fig.4). Of the 5 Arg residues of the molecule, one (position 37) is located in the second hydrophilic region, flanked by other charged residues, while the remaining 4 (positions 53, 55, 58 and 61) are clustered in the third hydrophilic region (hatched in fig.4).

To locate the fast-reacting Arg residue essential for DNA-binding within the BS-NS molecule, fingerprints of peptic peptides were obtained from the native, as well as from the 2,3-butanedione-reacted protein. After identification of the Arg-containing peptides (hatched in fig.5) these were

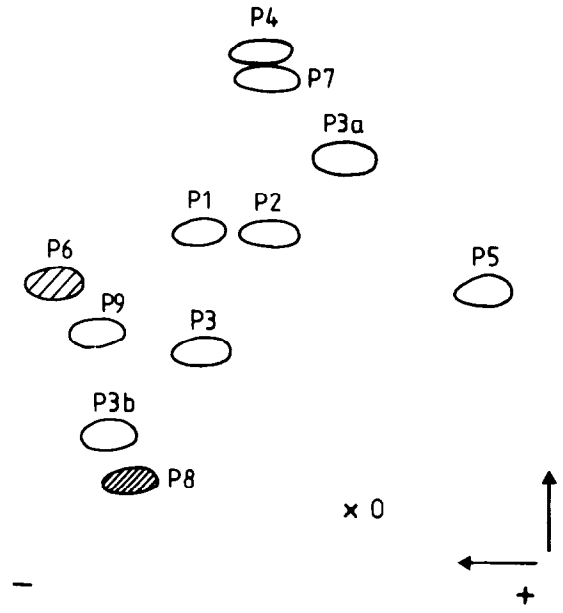

Fig.5. Peptic fingerprints of BS-NS. The conditions for the peptic hydrolysis and the peptide nomenclature were as in [7]. After location of all peptides by fluorescamine reaction [16] the Arg-containing peptides (hatched) were identified by the 9,10-phenanthroquinone reaction [17].

Table 1

Amino acid composition of Arg-containing peptic peptides of 2,3-butanedione-modified BS-NS

Reaction time (min)	DNA-binding activity (%)	Peptide[a]	Amino acid composition
0	100	P8:	Asx (1); Thr (1); Glx (5); Pro (1); Gly (2); Ala (2); Val (1); Lys (1); Arg (4)
		P6 + P9:	Asx (2); Ser (1); Glx (2); Pro (3); Gly (2); Ala (5); Val (3); Met (1); Ile (1); Leu (3); Phe (1); Lys (7); Arg (1)
20	11	P8:	Asx (1.6); Thr (1.1); Ser (1.3); Glx (5.4); Gly (4.1); Ala (2.7); Val (1.0); Pro (0.8); Lys (1.9); Arg (2.2)
		P6 + P9:	Asx (2.4); Ser (1.4); Glx (2.6); Pro (1.3); Gly (3.8); Ala (4.0); Val (2.3); Met (1.4); Ile (0.9); Leu (4.0); Phe (1.0); Lys (7.0); Arg (1.0)
50	5.7	P8:	Asx (1.6); Thr (1.2); Ser (0.7); Glx (5.1); Pro (0.6); Gly (4.0); Ala (1.8); Val (1.0); Met (0.6); Lys (1.8); Arg (1.0)
		P6 + P9:	Asx (2.3); Ser (1.5); Glx (2.4); Pro (1.3); Gly (3.6); Ala (3.8); Val (2.2); Met (1.4); Ile (1.0); Leu (3.9); Phe (1.0); Lys (7.0); Arg (0.9)

[a] The nomenclature of the BS-NS peptic peptides is as in [7]

extracted and their amino acid compositions determined. As seen in table 1, the Arg content of peptide P8 is drastically reduced (from 4 to 2.2 residues after 20 min), while Arg 37, located in peptide P6, does not react (or reacts very slowly) with butanedione, even after 50 min incubation. Thus, at least one residue of the 35–42 peptide of BS-NS is not exposed suggesting that this region of the molecule is not involved in DNA binding; this is also compatible with the fact that the arginine in this position is not conserved in other bacterial species [7]. The arginines contained in the 51–70 region, on the other hand, are exposed and at least one of them is required for DNA binding. Since this region of the molecule is one of the most conserved among the homologous proteins from other bacteria [7], it is tempting to speculate that it is this part of the molecule which is primarily responsible for the interaction with the DNA. Protection of the arginine residues of this region from modification in the presence of DNA (not shown) and loss of DNA binding capacity of *E. coli* NS-2 following the photooxidation of the single His residue (position 54) also localized in this region of the molecule (not shown) lend further support to the above-mentioned premises. The involvement of this peptide in DNA binding is also compatible with and suggested by the recently resolved 3-dimensional X-ray crystallographic structure of BS-NS at 3.0 Å resolution (Tanaka et al., in preparation).

ACKNOWLEDGEMENTS

We express our gratitude to Professor H.G. Wittmann for his interest and encouragement, to Professor F. Bohlmann and Dr J. Jakupovic (Institute of Organic Chemistry, TU-Berlin) for the use of the NMR spectrometer and to Mr K. Ashman for running the computer program.

REFERENCES

[1] Rouvière-Yaniv, J. and Gros, F. (1975) Proc. Natl. Acad. Sci. USA 72, 3428–3432.
[2] Varshavsky, A.J., Nedospasov, S.A., Bakayev, V.V., Bakayeva, T.G. and Georgiev, G.P. (1977) Nucleic Acids Res. 4, 2725–2745.
[3] Suryanarayana, T. and Subramanian, A.R. (1978) Biochim. Biophys. Acta 520, 342–357.
[4] Geider, K. and Hoffmann-Berling, H. (1981) Annu. Rev. Biochem. 50, 233–260.
[5] Lammi, M., Paci, M., Pon, C.L., Losso, M.A., Miano, A., Pawlik, R.T., Gianfranceschi, G.L. and Gualerzi, C.O. (1984) in: Proteins Involved in DNA Replication (Hübscher, U. and Spadari, S. eds) Plenum, New York, in press.
[6] Mende, L., Timm, B. and Subramanian, A.R. (1978) FEBS Lett. 96, 395–398.
[7] Kimura, M. and Wilson, K.S. (1983) J. Biol. Chem. 258, 4007–4011.
[8] Paci, M., Pon, C.L., Losso, M.A. and Gualerzi, C. (1984) Eur. J. Biochem. 138, 193–200.
[9] Rouvière-Yaniv, J. and Kjeldgaard, N.O. (1979) FEBS Lett. 106, 297–300.
[10] Dijk, J., White, S.W., Wilson, K.S. and Appelt, K. (1983) J. Biol. Chem. 258, 4003–4006.
[11] Miano, A., Losso, M.A., Gianfranceschi, G.L. and Gualerzi, C.O. (1982) Biochem. Int. 5, 415–422.
[12] Riordan, J.F. (1973) Biochemistry 12, 3915–3923.
[13] Lammi, M., Paci, M., Pon, C.L. and Gualerzi, C. (1982) Biochem. Int. 5, 429–436.
[14] Pon, C.L., Wittmann-Liebold, B. and Gualerzi, C. (1979) FEBS Lett. 101, 157–160.
[15] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[16] Vandekerckhove, J. and Van Montagu, M. (1974) Eur. J. Biochem. 44, 279–288.
[17] Yamada, S. and Itano, H.A. (1966) Biochim. Biophys. Acta 130, 538–540.