INTERACTION OF *lac* REPRESSOR FRAGMENT 33–38 (Lys–Thr–Arg–Glu–Lys–Val) WITH HOMO-OLIGONUCLEOTIDES

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1. Introduction

Based on model building it was proposed [1] that Arg–Glu sequences in polypeptides may selectively recognize guanine bases in double-stranded nucleic acids as well as guanine and cytosine bases in single-stranded nucleic acids. In particular, it was suggested that the sequence Arg 35–Glu 36–Lys 37 of *lac* repressor [2] may bind selectively to a GC base pair of the *lac* operator [3]. This hypothesis was based on the notion that the guanidino-group of arginine may form a pair of hydrogen bonds with guanine (or cytosine in single strands) and the adjacent carboxyl-group of glutamic acid may serve to compensate the positive charge of the guanidino-group and thus enhance the stability of the complex. The arguments in favour of this hypothesis are persuasive and thus we decided to test it by model studies using homo-oligonucleotides and a synthetic *lac* repressor fragment containing the Arg–Glu sequence. Here, the results obtained with the *lac* repressor fragment 33–38 (Lys–Thr–Arg–Glu–Lys–Val) are described. If the hypothesis was correct, even the simple model systems tested in this study should exhibit qualitative differences. However, no evidence was obtained for any clear specificity of interaction between the repressor fragment and various single- or double-stranded nucleic acids. The binding affinity of the repressor fragment appears to be correlated to the degree of base stacking in the oligonucleotides in a similar manner as observed for other oligopeptide–oligonucleotide complexes [4]. The rate of binding is controlled by diffusion.

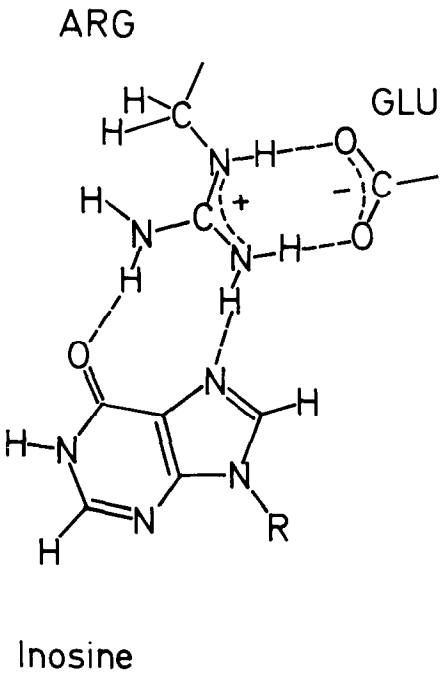

Fig.1. Hydrogen bonding scheme for the interaction of Arg–Glu with inosine (cf. [1]).

2. Materials and methods

2.1. *Peptide synthesis and purification*

The protonated N^{α}-amino group at Lys 33 and the carboxylate group at Val 38 of the repressor fragment Lys–Thr–Arg–Glu–Lys–Val are not present at the corresponding positions of the native repressor. However, Glu 39 may provide a negative charge in the identical position of native repressor.

The hexapeptide was synthesized by the solid

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phase method [5,6] starting with 0.12 mmol *t*-Boc-L-Val/750 mg protected amino acid resin and following the procedure in [7] except that 5% diisopropylethylamine in CH_2Cl_2 (v/v) was employed in the neutralization step. The peptide was deprotected and cleaved from the resin by anhydrous HF [7] and chromatographed on a Bio-Gel P-2 column. The yield of crude peptide was 60.4 mg (66.2%). Further purification was achieved by ion-exchange chromatography on CM-cellulose. The material with the correct amino acid composition was eluted between 0.5 M pyridine acetate (pH 5.55) and 1 M pyridine acetate (pH 6.20). After another chromatography step on Bio-Gel P-2 the yield of purified peptide was 30.8 mg (33.8%) based on the amount of COOH-terminal valine originally esterified to the resin.

The amino acid composition of the purified hexapeptide was Thr 1.0, Glu 1.0, Val 1.0, Lys 2.1, Arg 1.0. The synthetic *lac* repressor fragment was homogeneous on thin-layer electrophoresis at pH 3.4 and pH 6.4. It was also homogeneous on thin-layer chromatography in *n*-butanol, acetic acid, pyridine, water (15:3:10:12, R_F = 0.09).

Lys–Gly–Lys was obtained from Bachem Feinchemikalien AG, Switzerland.

2.2. *Oligonucleotides*

$A(pA)_5$, $A(pA)_7$, and $U(pU)_7$ were obtained from Boehringer Mannheim. $I(pI)_5$, $I(pI)_7$, $C(pC)_7$ and $d[(pA)_8]$ were purchased from Collaborative Research. The terminal phosphate of $d[(pA)_8]$ was removed by alkaline phosphatase and the resulting $d[(pA)_7]$ was purified by paper chromatography. The polymers poly[d(A–T)], poly[d(I–C)] and calf thymus DNA were obtained from Boehringer Mannheim.

2.3. *Methods*

The binding of the peptide to various oligonucleotides was analyzed by the field jump method in [4].

The field jump experiments were performed in 1 mM Na-cacodylate, 50 μM EDTA (pH 5.9). The melting curves were measured in 1 mM NaCl, 1 mM Na-cacodylate, 0.2 mM EDTA (pH 7.0).

3. Results and discussion

3.1. *Oligonucleotides*

Since the conformation of the repressor fragment is likely to be rather flexible, it is not possible to give a reliable estimate of the number of nucleotides which are covered by the fragment upon binding. For the present model studies the oligonucleotides were chosen to be sufficiently long for optimal binding contacts, but not long enough for multiple binding. This selection was based upon previous model investigations [4,8–10]. The results obtained demonstrated a 1:1 stoichiometry for all cases investigated. As expected, the binding of a second molecule of repressor fragment which was possible, e.g., in the case of octanucleotides, did not occur under the experimental conditions chosen; obviously the binding of one molecule of fragment decreased the affinity for the binding of a second molecule, e.g., by lowering the electrostatic potential and by excluding binding sites. The binding parameters were obtained from the concentration dependence of relaxation amplitudes and time constants (cf. fig.2). The results are compiled in table 1. According to the hypothesis proposed in [1] the repressor fragment should interact preferentially with guanine nucleotides. This interaction, however, could not be studied directly since field jump experiments using $G(pG)_7$ failed to show sufficiently large amplitudes. To evade these difficulties caused mainly by the strong tendency of oligo(G) and poly(G) to aggregate [11], oligo(I)s were used instead of oligo(G)s since inosine provides a hydrogen-bonding acceptor site corresponding to that of guanosine.

The highest binding constants K were observed for oligoriboadenylates. The K-values obtained for $I(pI)_n$

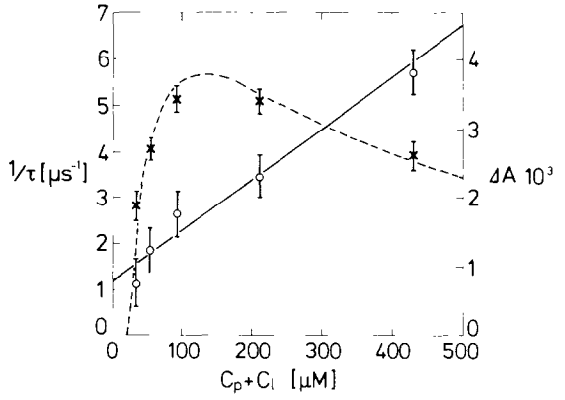

Fig.2. Reciprocal relaxation times $1/\tau$ (○) and amplitudes ΔA (×) for $U(pU)_7$ + repressor fragment as a function of the free reactant concentration $c_p + c_l$ (measured at a constant 20.9 μM $U(pU)_7$; equilibrium constant determined from amplitudes and used for fitting of $1/\tau$ values).

Table 1
Equilibrium constants K and forward rate constants k_R for the complex formation of the repressor fragment with various oligonucleotides

	K (mM^{-1})	k_R ($M^{-1}\ s^{-1}$)
$A(pA)_7$	17.0	$8.2 \cdot 10^9$
$d[A(pA)_7]$	2.9	–
$I(pI)_7$	10.0	–
$U(pU)_7$	9.3	$1.1 \cdot 10^{10}$
$C(pC)_7$	7.7	–
$A(pA)_5$	4.3	–
$I(pI)_5$	3.5	–

of comparable chain length n were clearly lower. Furthermore, the repressor fragment did not show any preferred interaction with $C(pC)_7$. The series of binding constants was similar to that observed for other simple oligopeptide–oligonucleotide complexes [4]. Purine nucleotides usually have higher affinity for peptides than pyrimidine nucleotides. The affinity may be related to the degree of stacking and thus to the degree of order which is expected to have a strong influence on the electrostatic potential. The deviating result obtained with $d[A(pA)_7]$ cannot be explained. Rate constants were evaluated only for $A(pA)_7$ and $U(pU)_7$. In both cases the rate of complex formation was determined by diffusion. The same mechanism of formation was observed for various other oligopeptide–oligonucleotide complexes [4].

3.2. *Double-stranded polymers*

Interaction between single-stranded nucleotides and repressor fragment could be studied by field jump experiments because the absorbance changes of the nucleotide caused by fragment binding were sufficiently large for a quantitative analysis. In vivo, however, repressor binds to double-stranded DNA. Since the absorbance change of double-stranded nucleic acids was too small, their interaction with the repressor fragment was probed by an analysis of the thermal helix–coil transition. Addition of the repressor fragment increased the temperature T_m corresponding to the midpoint of the helix–coil transition. This increase was measured at various peptide concentrations using 3 different double helices. When the T_m-values were plotted as a function of the logarithm of the peptide concentration, straight lines were obtained. The slopes and the increase of the melting temperatures found in the presence of 95 μm peptide are given in table 2. The parameters obtained for the repressor fragment showed a preferential stabilisation of the poly[d(A–T)] double helix indicating a higher binding affinity. However, the parameters obtained for the tripeptide Lys–Gly–Lys were very similar. Thus the effects caused by the repressor fragment were most likely not due to its particular Arg–Glu sequence but apparently depended on differences of the charge spacing in the double helices.

3.3. *General conclusion*

A short fragment of a complex protein in solution is not expected to have the same conformation as in its native environment. Thus it cannot be excluded that the absence of specific interactions between the repressor fragment and various oligo- and polynucleotides is due to the fact that a particular conformation of the fragment required for specificity is not accessible or sufficiently stable [12]. However, if the interaction between the Arg–Glu sequence and guanosine or inosine would be strong, it should be possible to arrange the peptide conformation for the appropriate

Table 2
Increase of the melting temperature T_m upon addition of the repressor fragment to various double helices – Data for Lys–Gly–Lys are given for comparison

	$dT_m/d \log c$		ΔT_m (0–95 μM)	
Polymer	Repressor fragment	Lys–Gly–Lys	Repressor fragment	Lys–Gly–Lys
d(A . T)	16.7	17.2	22.4	22.4
d(I . C)	13.0	14.5	21.4	20.5
DNA	13.1	14.4	17.4	16.2

$dT_m/d \log c$ is determined over 10–100 μM peptide. ΔT_m gives the increase in the melting temperature after addition of repressor fragment from 0–95 μM

molecular contacts. These results demonstrate that the specificity of the Arg–Glu segment to bind to nucleic acids is not as high as was expected from model building.

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