THERMODYNAMIC AND KINETIC STUDIES OF THE INTERACTION BETWEEN T5-PHAGES AND THEIR RECEPTOR, ISOLATED FROM *ESCHERICHIA COLI* B, IN THE ABSENCE AND PRESENCE OF TRITON X-100

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ABSTRACT Particles with receptor activity for T5-phages were isolated from the outer membrane of *Escherichia coli* B. We describe the interaction of these particles with T5-phages as a two-step chemical reaction. The rate constants were estimated from the inactivation kinetics. The transition-state theory permits the calculation of the entropy, enthalpy, and Gibbs free energy of activation. In the absence of Triton X-100, the reaction can be described with one set of thermodynamic constants for the temperature range from 10° to 40°C. The addition of Triton, which results in the splitting of receptor particles and in the building of mixed micelles, causes a complicated dependence on temperature. In this case, a subdividing of the temperature range measured into two parts yields two sets of thermodynamic constants that permit a good description of experimental kinetics.

I. INTRODUCTION

The interaction between intact bacteria and bacteriophages has been studied intensively (Puck et al., 1951; Garen and Puck, 1951; Stent and Wollman, 1952; Garen, 1954; Christensen, 1965; Watanabe et al., 1980). However, very little is known about the reaction of phages with isolated receptors. The first thermodynamic approach to the inactivation process of T5-phages by isolated receptors was performed by Zarnitz and Weidel (1963) under the assumption of a pseudo monomolecular irreversible reaction.

Zarybnicky et al. (1973) have shown that the whole DNA of the T5-phage is ejected in one step after the adsorption to the receptor in vitro; this contrasts with a two-step injection process in vivo, found by Lanni (1965).

We had studied (Zarybnicky et al., 1980) the adsorption kinetics at incubation times up to 10 h, which result in high inactivation of phages (up to $p/p_0 = 10^{-5}$), and found that the phage titer decreases more slowly with time than expected for the ideal irreversible bimolecular or pseudomonomolecular reaction. We explain this deviation by introducing a reversible step similar to the model for the adsorption of T1-phages to bacteria as described by Garen (1954) and Christensen (1965). A reversible inactivation step was also found by Schwartz (1975) for the reaction between λ-phage and its isolated receptor.

Our model assumes the formation of a dissociable phage-receptor complex, which may either decay back to free phage and receptor or proceed to an irreversible complex. The formation of the irreversible complex is necessary for the ejection of the DNA. The

ghost-receptor complex remaining after DNA ejection is stable as well. This means that the receptor does not work like an enzyme and cannot inactivate several phages successively.

The time behavior of this process can be predicted from the system of differential equations according to the reaction model. In general, this system can be solved only numerically. However, an explicit solution can be found for the case that receptors are in great excess over phages.

The rate constants, which determine the shape of the theoretically derived inactivation curves, can be estimated with good accuracy by fitting these theoretical curves to the experimental data using the mathematical method of least squares. A good conformity had been found for different receptor-to-phage ratios, even for those lower than one (Zarybnicky et al., 1980).

The present paper deals with the temperature dependence of the inactivation process between intact receptor particles, prepared as described by Weidel (1954), and the T5-phage. The rate constants of the reaction steps in the temperature range from 10° to 40°C were estimated. We used the transition-state theory for reaction kinetics to determine the thermodynamic data $\Delta H_i^{\ddagger}$, $\Delta S_i^{\ddagger}$, $\Delta G_i^{\ddagger}$, $\Delta H_{1,2}$, $\Delta S_{1,2}$, and $\Delta G_{1,2}$. The inactivation curves that were calculated directly from these thermodynamic constants coincide very well with the experimental data. Additionally, the influence of Triton X-100 on the inactivation process and on the splitting of the receptor particles is discussed.

II. MATERIALS AND METHODS

(a) Chemicals

Except for Triton X-100, which was from Packard Instrument Company, Inc. (Downers Grove, Ill.), and the Good buffers, which were from Serva GmbH & Co. (Heidelberg, F.R.G.), all chemicals were from Merck (Darmstadt, F.R.G.). All but Triton X-100 were analytical grade. Bio-Gel A-50m and A-1.5m were from Bio-Rad Laboratories (Richmond, Calif.). [U-^{14}C] protein hydrolysate for the labeling of T5-receptor was obtained from Amersham Buchler GmbH & Co. kG (Braunschweig, F.R.G.).

(b) Bacteria and Bacteriophages

Escherichia coli B was originally a gift from Dr. Kellenberger. The phage T5st was from the collection of the late Dr. Weidel. The phage stocks we used were prepared as described (Zarybnicky et al., 1973), and purified by CsCl gradient centrifugation. *Escherichia coli* B for receptor preparations were grown in a synthetic culture medium, containing 1.9 g/liter NaCl, 1.25 g/liter NH_4Cl, 0.15 g/liter $MgSO_4 \cdot 7H_2O$, 0.075 g/liter $CaCl_2 \cdot 2H_2O$, 10 g/liter sodium lactate, 4.25 g/liter glycerol, 1.875 g/liter KH_2PO_4, 18.24 g/liter $Na_2HPO_4 \cdot 12H_2O$, 0.25 g/liter aspartic acid, and 0.25 g/liter glutamic acid, at pH 7.4. For the preparation of radioactively labeled T5-receptors the two amino acids were omitted.

(c) Preparation of T5-Receptors

T5-receptor was isolated from *E. coli* B as described by Weidel et al. (1954), except that we used no pancreatin treatment and no acetic acid precipitation. Additionally, the receptor was purified by differential centrifugation in a Sorvall GSA rotor (Ivan Sorvall, Inc., Norwalk, Conn.) (twice at 10,000 *g* for 40 min and 16,300 *g* for 24 h, and once 10,000 *g* for 40 min, and 16,300 *g* for 60 h, at 4°C). Residual membrane debris and contaminating low molecular weight components were separated according to Braun et al. (1973) by two passages over a preparative Bio-Gel A-50m (100–200 mesh) column (2.6 × 58.0 cm) which was equilibrated with 10 mmol/liter phosphate buffer of pH 7.4 and 0.02% NaN_3. The fractions inactivating T5-phages and free from membrane debris were combined and

concentrated by centrifugation in a Sorvall GSA rotor (4°C, 16,300 *g* for 60 h). The pellet was washed once in a solution of 0.02% NaN_3 in bidistilled water, centrifuged (Spinco Type 35 rotor [Beckmann Instruments, Spinco Div., Palo Alto, Calif.], 4°C, 100,000 *g* for 10 h), and redissolved in the washing solution. From 70 g of *E. coli* B we got 14 ml of this receptor stock solution containing 0.55 mg/ml of protein, determined according to Lowry et al. (1951) with a bovine serum albumin calibration curve, and 0.44 mg/ml of lipopolysaccharide, determined as 2-keto-3-deoxyoctonic acid according to Karkhanis et al. (1978) against a lipopolysaccharide standard isolated from the bacteria strain used. The receptor preparation had a specific activity of $(1.0 \pm 0.1)\ 10^{13}$ receptor units (RU)/mg protein (for definition and estimation see Kinetic Experiments).

Radioactively labeled T5-receptors were prepared similarly. To 5 liters of a well-aerated culture of *E. coli* B with a titer of 2.5×10^6 bacteria/ml, 0.05 mCi of [U-^{14}C]-protein hydrolysate were added. The bacteria were harvested at a titer of 1.0×10^9 bacteria/ml. The final [^{14}C] T5-receptor stock solution contained 0.7 mg/ml of protein and 0.56 mg/ml of lipopolysaccharide. The incorporated radioactivity was 39,000 dpm/mg of protein.

(d) Analytical Methods

The Lowry method (Lowry et al., 1951) and the lipopolysaccharide determination (Karkhanis et al., 1978) are heavily disturbed by the presence of Triton X-100 (Hartree, 1972; Gaál and Holczinger, 1975; Petterson, 1979). Our standard Triton X-100 concentration was 0.3%. In the Lowry method, an easily sedimenting precipitate is formed during color development, and can be eliminated by centrifugation in a tabletop centrifuge. The test remains linear up to 0.5 mg/ml of protein. The extinction of the blank (compared with a blank without Triton) at a wavelength of 690 nm increases to 0.1/cm.

Similarly, in the lipopolysaccharide determination method, the red-brown precipitate that is formed after addition of the $NaAsO_2$ reagent, can be separated. In this case, the samples for the calibration curve may remain without Triton X-100, as the specific color development does not change.

The radioactivity of the fractions of the analytical Bio-Gel separations was counted in Quickszint 402 scintillator solution (Zinsser, Frankfurt, F.R.G.) (1 ml sample plus 9 ml scintillator solution) with a Packard 3320 Tri-Carb scintillation spectrometer.

(e) Kinetic Experiments

The receptor dilutions in incubation buffer with 0.3% and without Triton were prepared several weeks before the experiment in order to equilibrate the dissociation of large receptor clusters.

For the kinetic experiments at different temperatures we added 0.1 ml of the appropriate receptor dilution to 1.8 ml Tris-HCl buffer of pH 7.7 with 0.3% or without Triton. The mixture was preincubated for 1 h before the addition of the phages. The addition of 0.1 ml of T5-phage stock suspension yielded 2.0 ml of sample with a receptor concentration of 3×10^{10} RU/ml, a phage titer of 2×10^8 plaque forming units (PFU)/ml, 10 mmol/liter buffer (pH 7.7), 10 mmol/liter of NaCl, and, if present, 0.3% Triton. The final concentration of divalent cations was $<10^{-6}$ mol/liter. All solutions were prepared in bidistilled water. Samples were taken every hour after the addition of the phages, up to 8 h. After appropriate dilution in cold 0.01% gelatin, 100 mmol/liter NaCl, 1 mmol/liter $MgCl_2$, 1 mmol/liter $CaCl_2$, 10 mmol/liter Tris-HCl (pH 7.4) solution the surviving PFU were assayed as follows, in a variation of the classical Adams method (Adams, 1966). A sample of 0.1 ml of the phage dilution plus 0.5 ml of *E. coli* B suspension (5×10^8/ml) was added to 5 ml of soft agar at 46°C (0.1 mol/liter NaCl, 1 mmol/liter $MgCl_2$, 1 mmol/liter $CaCl_2$, 1% Difco Bacto-Tryptone (Difco Laboratories, Detroit, Mich.), 0.44% Difco Bacto-Agar, pH 7.3), and the phage/bacteria/agar-mixture was plated on a 94-mm plastic petri dish after mixing (Zarybnicky et al., 1980). This method shows better reproducibility than the classical agar bilayer method described by Adams. For better accuracy, all samples were plated four times; the mean values and the standard deviations were calculated.

The amount of receptor protein that can inactivate one phage is defined as one unit of specific receptor activity (RU). RU was measured by incubating receptor at high concentration r_0 with an excess of phages p_0 at 35°C. The optimum ratio of r_0/p_0 was between 0.3 and 0.7. With the receptor

concentrations used ($\sim 10^{10}$ RU/ml), the final phage titer was reached after ~2 h at 35°C. It did not change for 24 h.

III. RESULTS

(a) Kinetics of Phage Inactivation

As described by Zarybnicky et al. (1980), the inactivation of T5-phages by isolated T5-receptors in vitro differs from a bimolecular irreversible reaction for long incubation times and high inactivations.

We explained this deviation successfully by introducing a reversible reaction step to a dissociable phage-receptor complex ⟨PR⟩ followed by an irreversible step, yielding a phage-receptor complex [PR] which can no longer dissociate to free phage and receptor. The complex [PR] decays under certain conditions to a stable ghost-receptor complex [GR] and free DNA (Zarybnicky et al., 1973):

$$\mathrm{P} + \mathrm{R} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \langle \mathrm{PR} \rangle \xrightarrow{k_3} [\mathrm{PR}] \xrightarrow{k_4} [\mathrm{GR}] + \mathrm{DNA}. \tag{1}$$

This reaction is described by the following system of nonlinear differential equations:

$$\mathrm{d}p/\mathrm{d}t = -k_1 \cdot p \cdot r + k_2 \cdot \langle pr \rangle \tag{2a}$$

$$\mathrm{d}\langle pr \rangle/\mathrm{d}t = k_1 \cdot p \cdot r - (k_2 + k_3) \cdot \langle pr \rangle \tag{2b}$$

$$\mathrm{d}[pr]/\mathrm{d}t = k_3 \cdot \langle pr \rangle - k_4 \cdot [pr] \tag{2c}$$

$$\mathrm{d}DNA/\mathrm{d}t = k_4 \cdot [pr] \tag{2d}$$

where p, r, $\langle pr \rangle$, $[pr]$, and DNA are the concentrations of phages, receptor units, dissociable and irreversible phage-receptor complexes, and DNA, respectively.

This system has an explicit solution only if the receptor concentration r can be treated as constant with time, and equal to the initial receptor concentration r_0. This means that receptor units have to be in great excess over phages. Under this assumption ($r_0 >> p_0$), we obtain the following equations as the solution of the system of Eqs. 2:

$$p/p_0 = A_1 \cdot e^{-\lambda_1 \cdot t} + A_2 \cdot e^{-\lambda_2 \cdot t} \tag{3a}$$

$$\langle pr \rangle/p_0 = B_1 \cdot e^{-\lambda_1 \cdot t} + B_2 \cdot e^{-\lambda_2 \cdot t} \tag{3b}$$

$$[pr]/p_0 = C_1 \cdot e^{-\lambda_1 \cdot t} + C_2 \cdot e^{-\lambda_2 \cdot t} - (C_1 + C_2) \cdot e^{-k_4 \cdot t} \tag{3c}$$

$$DNA/p_0 = 1 - k_4 \cdot \left(\frac{1}{\lambda_1} \cdot C_1 \cdot e^{-\lambda_1 \cdot t} + \frac{1}{\lambda_2} \cdot C_2 \cdot e^{-\lambda_2 \cdot t} \right) + (C_1 + C_2) \cdot e^{-k_4 \cdot t} \tag{3d}$$

where

$$\lambda_{1,2} = \frac{1}{2} \left[(k_1 \cdot r_0 + k_2 + k_3) \pm \sqrt{(k_1 \cdot r_0 + k_2 + k_3)^2 - 4k_1 \cdot r_0 \cdot k_3} \right] \tag{4}$$

and the time-independent cofactors are given by

$$A_1 = \frac{k_1 \cdot r_0 - \lambda_2}{\lambda_1 - \lambda_2} \qquad A_2 = 1 - A_1 \tag{5a}$$

$$B_1 = \frac{(k_1 \cdot r_0 - \lambda_1) \cdot (k_1 \cdot r_0 - \lambda_2)}{k_2 \cdot (\lambda_1 - \lambda_2)} \qquad B_2 = -B_1 \tag{5b}$$

$$C_1 = \frac{k_3 \cdot (k_1 \cdot r_0 - \lambda_1) \cdot (k_1 \cdot r_0 - \lambda_2)}{k_2 \cdot (k_4 - \lambda_1) \cdot (\lambda_1 - \lambda_2)} \qquad C_2 = -\frac{k_4 - \lambda_1}{k_4 - \lambda_2} \cdot C_1. \tag{5c}$$

The values calculated by means of these equations differ only slightly from the values that were obtained by numerically solving the system of Eqs. 2 if $r_0/p_0 > 10$ RU/PFU. In the experiments presented in this paper, a ratio of $r_0/p_0 = 150$ RU/PFU was used.

(b) Determination of the Thermodynamic Constants

The mathematical analysis of the measured inactivation values enables us to estimate the rate constants k_1, k_2, and k_3 with good accuracy.

Using the transition-state theory, originally developed by Eyring (1935), we can express the rate constants, k_i, as functions of the absolute temperature, T, the entropy of activation, $\Delta S_i^{\ddagger}$, and the enthalpy of activation, $\Delta H_i^{\ddagger}$, in the following form

$$k_i = \frac{k \cdot T}{h} \cdot e^{\Delta S_i^{\ddagger}/R} \cdot e^{-\Delta H_i^{\ddagger}/R \cdot T} \tag{6}$$

where k, h, R, and T are the Boltzmann, Planck, and the gas constants, and the absolute temperature, respectively. Substituting

$$q_i = \frac{k}{h} \cdot e^{\Delta S_i^{\ddagger}/R} \tag{7}$$

into Eq. 6, yields

$$\frac{k_i}{T} = q_i \cdot e^{-\Delta H_i^{\ddagger}/R \cdot T}. \tag{8}$$

Application of the mathematical method of exponential regression (see Appendix) to the values of k_i/T obtained by least-squares fits of Eq. 3a to the experimental inactivation values allows the determination of $\Delta H_i^{\ddagger}$ and $\Delta S_i^{\ddagger}$.

Comparing $\mathrm{d}(\ln k_i)/\mathrm{d}T$ of the theoretical transition-state expression (Eq. 6) with $\mathrm{d}(\ln k_i)/\mathrm{d}T$ of the empirical Arrhenius expression

$$k_i = A \cdot e^{-E_A/R \cdot T} \tag{9}$$

which neglects the linear dependence on the absolute temperature, gives

$$E_A \cong \Delta H_i^{\ddagger} + R \cdot T \tag{10}$$

for the Arrhenius energy of activation. It varies with temperature to some extent in contrast to the enthalpy of activation, which is temperature independent. In as much as $R \cdot T$ at room

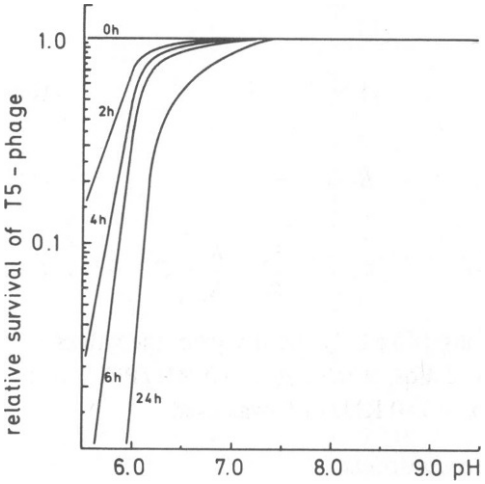

FIGURE 1

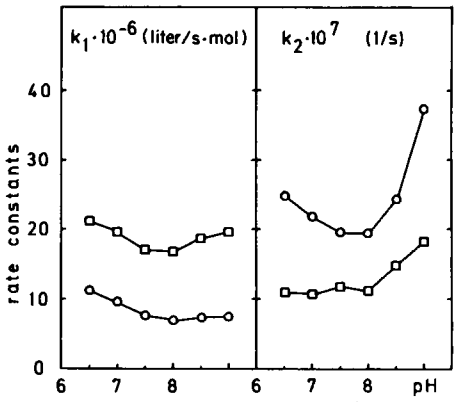

FIGURE 2

FIGURE 1 Time stability of T5-phages in dependence on pH at 35°C. The media contained 10 mmol/liter of NaCl, and were buffered with 100 mmol/liter of Tris-MES, Tris-TES, Tris-TABS for the pH ranges 5.5 to 6.5, 6.5 to 9.0, and 9.0 to 9.5, respectively.

FIGURE 2 pH dependence of the rate constants k_1 and k_2 at 35°C for the same media as described in Fig. 1, in absence (○) and in presence (□) of 0.3% Triton X-100.

temperature is ~2.4 kJ/mol, there is only a small difference between the empirical activation energy, E_A, and the enthalpy of activation, ΔH_i, of the transition-state theory.

(c) Influence of pH

To find an appropriate pH for our experiments, we incubated T5-phages at different pH at 35°C, and measured the decrease of the titer for up to 24 h. The media contained 10 mmol/liter NaCl, and were buffered within the pH-ranges of 5.0–6.5, 6.5–9.0, and 9.0–9.5 with 100 mmol/liter Tris-TES[1], Tris-TAPS, and Tris-MES, respectively. The concentration of all divalent cations was 10^{-3} mmol/liter. Fig. 1 gives the change in phage titer in the absence of Triton X-100 at different pH and incubation times up to 24 h. The addition of 0.3% of Triton caused no effect. In the pH range 7.5–9.5, the phage titer remained constant for 24 h. We also measured the inactivation of T5-phages by isolated receptor as a function of the pH in the same buffers as described above for the stability measurement. The rate constants k_1 and k_2 are shown in Fig. 2. The values of k_1 for pH < 7.0 are corrected for self-decomposition due to the low pH (see Fig. 1). We used a pH of 7.7 for all further experiments.

(d) Thermodynamic Studies of the Inactivation Kinetics

To determine the thermodynamic constants of the phage-receptor reaction, we measured the inactivation between 10° and 40°C in steps of 5°C. The temperatures during the preincuba-

[1]TES, 2-([Hydroxy-1,1-bis(hydroxymethyl)ethyl]amino)ethane sulfonic acid; TAPS, [2-Hydroxy-1,1-bis(hydroxymethyl)ethyl]amino)-1-propane sulfonic acid; MES: 2-[*N*-Morpholino]ethane sulfonic acid.

tion and the experiment were kept constant within 0.1°C. Phage and receptor stock suspensions were pipetted with an accuracy better than 1%. We used a Tris-HCl buffer concentration of 10 mmol/liter, pH 7.7. This concentration was sufficient to keep the pH constant within ±0.1, measured before and after the experiment. The ionic strength influences the inactivation rate of the phage-receptor reaction (data not shown). Highest inactivation rates were found for ionic strengths near zero, but the results were of poor reproducibility. Therefore we chose a concentration of 10 mmol/liter of NaCl. To exclude the influence of additional Mg^{2+}, which decreases the inactivation rate much more than Na^{+}, we used NaCl of superpure grade.

The 0.1 ml samples taken at 1-h intervals were diluted and plated immediately to stop the reaction. The measured relative survival of phages is given by the symbols in Fig. 3. Kinetics in the absence of Triton X-100 were quite reproducible. Repeated experiments gave identical results within the experimental errors.

Because the ratio r_0/p_0 was high in all experiments (~150 RU/PFU), we could use the

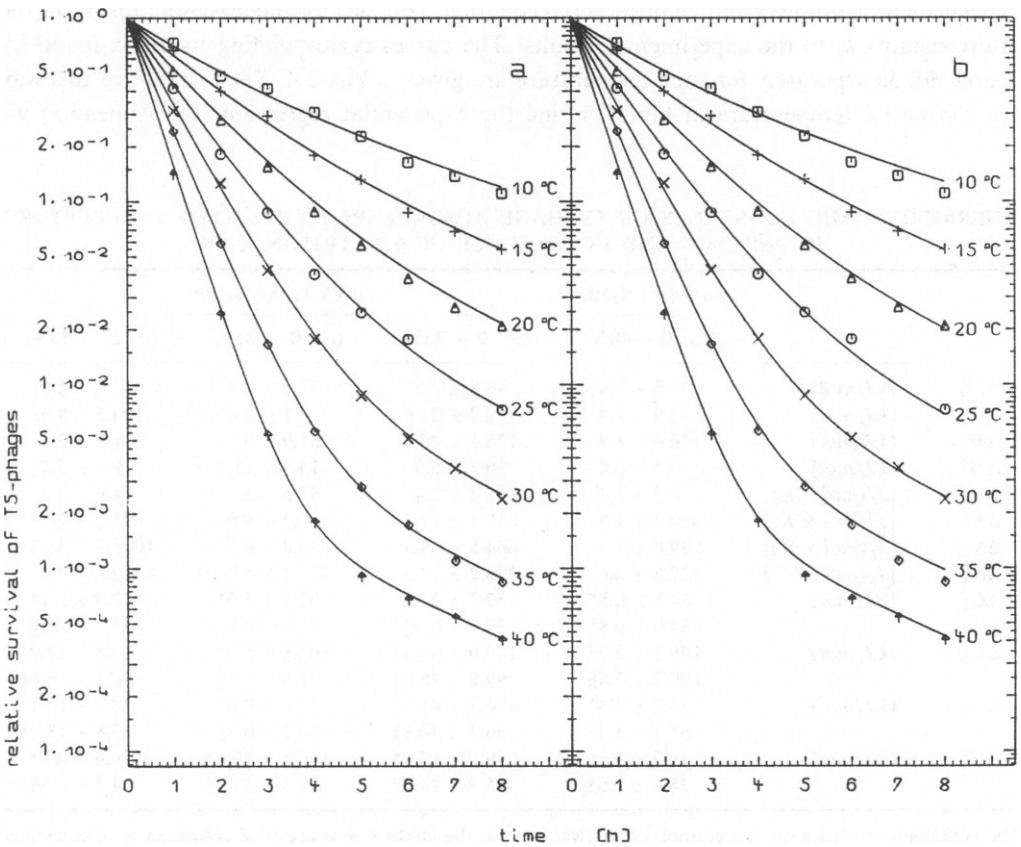

FIGURE 3 Inactivation of T5-phages by their receptors in absence of Triton X-100. The symbols in (*a*) and (*b*) represent identical experimental values. (*a*) compares these values with curves calculated from the rate constants k_1,k_2, and k_3 obtained by least-squares fitting for each temperature separately. (*b*) shows curves calculated from the thermodynamic data $\Delta H_1^{\ddagger}$, $\Delta H_2^{\ddagger}$, $\Delta H_3^{\ddagger}$, $\Delta S_1^{\ddagger}$, $\Delta S_2^{\ddagger}$, and $\Delta S_3^{\ddagger}$ obtained by exponential regression of the k_i from (*a*). The data are given in Table I*a*.

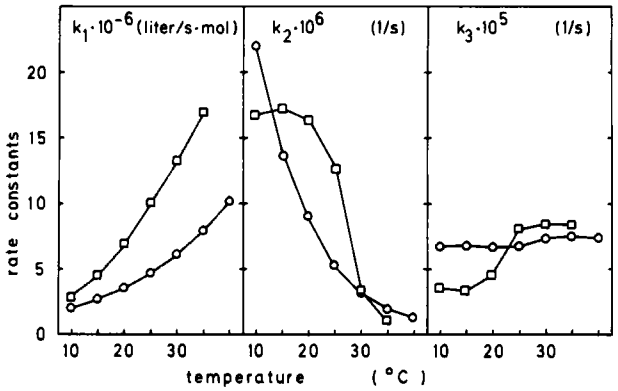

FIGURE 4 Temperature dependence of the rate constants k_1 to k_3 calculated from the experiments without (○) and with (□) 0.3% of Triton X-100.

explicit solution of the system of differential equations (Eq. 3a) for the least-squares fits of the rate constants k_i to the experimental results. The curves corresponding to the k_i found by fitting Eq. 3a separately for each temperature are given in Fig. 3*A*. The k_i obtained this way are plotted vs. temperature in Fig. 4. Using the exponential regression (see Appendix) we

TABLE I
THERMODYNAMIC CONSTANTS OF T5-PHAGE ADSORPTION TO ISOLATED T5-RECEPTORS IN ABSENCE AND IN PRESENCE OF 0.3% TRITON X-100

		no Triton X-100	0.3% Triton X-100		
		(*a*) 10 – 40°C	(*b*) 10 – 35°C	(*c*) 10 – 20°C	(*d*) 25 – 35°C
$\Delta H_1^{\ddagger}$	(*kJ/mol*)	37.5 ± 0.4	48.8 ± 2.5	57.8 ± 2.4	37.4 ± 1.0
$\Delta H_2^{\ddagger}$	(*kJ/mol*)	−72.9 ± 1.4	−79.7 ± 22.9	−4.1 ± 2.6	−194.5 ± 8.4
$\Delta H_{1,2}$	(*kJ/mol*)	110.4 ± 1.7	128.5 ± 20.5	61.9 ± 0.1	231.9 ± 9.5
$\Delta H_3^{\ddagger}$	(*kJ/mol*)	0.5 ± 0.8	30.3 ± 6.9	14.5 ± 15.3	0.1 ± 2.4
$\Delta S_1^{\ddagger}$	[*J/(mol × K)*]	8.7 ± 1.3	52.3 ± 8.4	83.6 ± 8.3	14.6 ± 3.4
$\Delta S_2^{\ddagger}$	[*J/(mol × K)*]	−590.3 ± 4.8	−612.1 ± 77.5	−350.2 ± 8.8	−991.0 ± 27.8
$\Delta S_{1,2}$	[*J/(mol × K)*]	599.0 ± 5.6	664.5 ± 69.3	433.8 ± 0.5	1005.3 ± 31.3
$\Delta S_3^{\ddagger}$	[*J/(mol × K)*]	−322.8 ± 2.6	−223.7 ± 23.3	−279.1 ± 53.0	−322.8 ± 7.7
$\Delta G_1^{\ddagger}$	(*kJ/mol*)	34.8 ± 0.8*	32.7 ± 5.1*	32.1 ± 5.0*	32.9 ± 2.1*
		35.0 ± 0.8§	33.5 ± 4.9§	33.3 ± 4.9§	33.1 ± 2.0§
$\Delta G_2^{\ddagger}$	(*kJ/mol*)	109.1 ± 2.9*	109.0 ± 46.8*	103.8 ± 5.3*	110.8 ± 17.0*
		100.2 ± 2.8§	99.8 ± 45.6§	98.6 ± 5.3§	96.1 ± 16.6§
$\Delta G_{1,2}$	(*kJ/mol*)	−74.2 ± 3.4*	−76.3 ± 41.9*	−71.8 ± 0.3*	−78.0 ± 19.1*
		−65.2 ± 3.3§	−66.3 ± 40.8§	−65.2 ± 0.3§	−62.8 ± 18.7§
$\Delta G_3^{\ddagger}$	(*kJ/mol*)	99.9 ± 1.6*	99.2 ± 14.1*	100.5 ± 31.6*	99.6 ± 4.7*
		95.1 ± 1.5§	95.9 ± 13.7§	96.3 ± 30.8§	94.7 ± 4.6§

The enthalpies of activation, the entropies of activation, and the Gibbs free energies of activation were calculated from the rate constants k_1, k_2, and k_3, using the exponential regression (see Appendix) in the given temperature range. The rate constants were obtained by least-squares fitting of Eq. 3a to the experimental values. The thermodynamic constants of the first reaction step, $\Delta H_{1,2}$, $\Delta S_{1,2}$, and $\Delta G_{1,2}$, were calculated from the corresponding equilibrium constant $K_{1,2} = k_1/k_2$. The definition for the standard error is given in the Appendix.

*Values calculated for 35°C.

§Values calculated for 20°C.

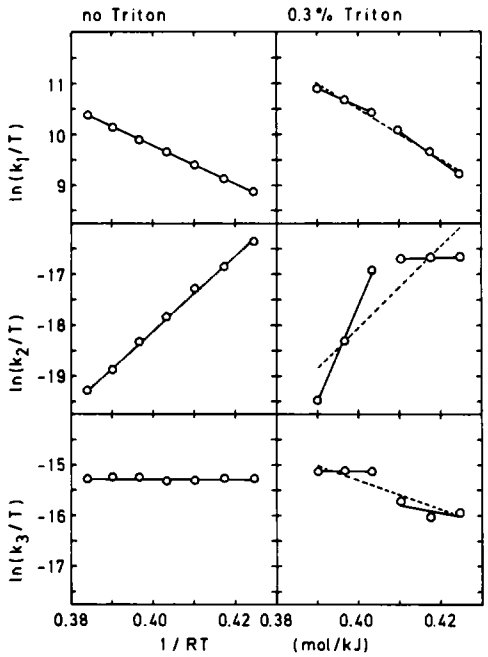

FIGURE 5 Plots of ln (k_i/T) vs. $1/RT$ for experiments without and with Triton X-100. The symbols give the values obtained from least-squares fits separately for each temperature. The solid lines on the left and the dashed lines on the right give the linear regression of these values. The solid lines on the right give the linear regression in the temperature ranges from 10° to 20°C and 25° to 35°C.

calculated the values for the enthalpies of activation and the entropies of activation from these k_i (see Table I*a*). The values of the changes in the enthalpy ($\Delta H_{1,2}$), the entropy ($\Delta S_{1,2}$), and the Gibbs free energy ($\Delta G_{1,2}$) for the first reaction step in Table I were calculated by using the exponential regression from the equilibrium constants $K_{1,2} = k_1/k_2$. The inactivation curves for these thermodynamic constants which were calculated from Eqs. 3a and 6 are compared with the measured values in Fig. 3*B*. A plot of $\ln(k_i/T)$ vs. $1/(R \cdot T)$ (in contrast to ln k_i vs. $1/T$ in the Arrhenius plot) in Fig. 5 demonstrates that the reaction follows the kinetics described by the eqs. 3a and 6 over the entire temperature range used.

(e) Effect of Triton X-100 on the Inactivation Process

As we found previously (Zarybnicky et al., 1980), the presence of 0.3% Triton X-100 increases considerably the initial relative inactivation rate $k_1 \times r_0$ of T5-phages by receptors. To learn something about the influence of Triton X-100 we studied the behavior of the receptor by gel chromatography on Bio-Gel A-50m. In all runs described, the columns were equilibrated with the elution buffer, and the samples were applied in the same buffer after a preincubation at the appropriate temperature for at least 1 h. In the absence of Triton X-100, the elution volume is independent of the buffer used (Fig. 6). Even the addition of 5 mmol/liter EDTA, which could result in the release of lipopolysaccharide (e.g. Leive et al., 1968), did not change the elution volume of the protein peak. The addition of 0.3% Triton X-100 results in disaggregation of the receptor particles (Fig. 7). The homogeneity of the

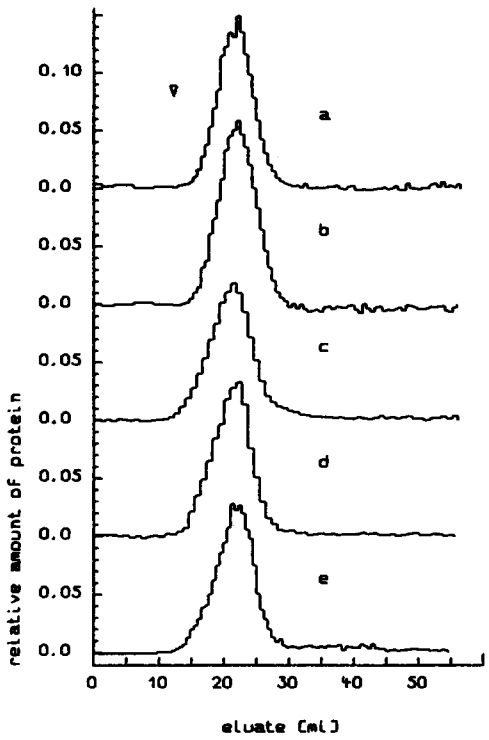

FIGURE 6

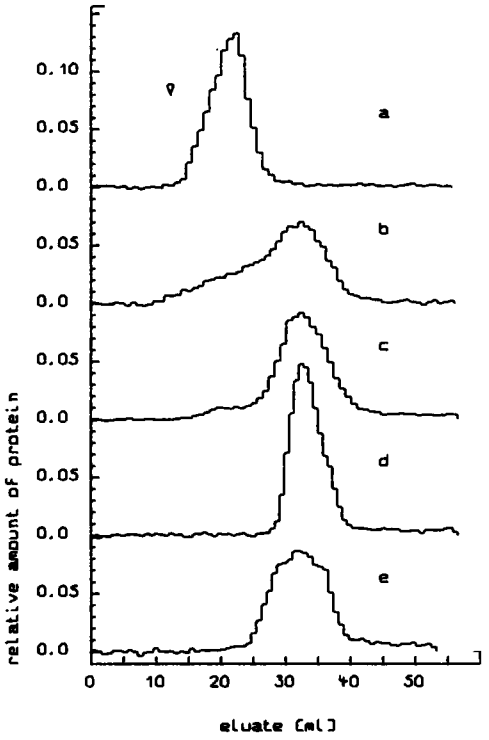

FIGURE 7

FIGURE 6 Behavior of purified T5-receptor preparations on Bio-Gel A-50m columns (0.9 × 58.0 cm) at 15°C in different buffer systems in the absence of Triton X-100. The protein concentrations were determined by the Fluram method (Udenfried et al., 1972) (*a*, *b*), or by liquid scintillation counting of ^{14}C-labeled protein (*c*, *d*, *e*). In all cases 0.032 mg of protein was applied in 0.05 ml of elution buffer, and 0.02% of NaN_3 was present. The triangle mark indicates the void volume. (*a*) 10 mmol/liter of phosphate buffer pH 7.4. (*b*) 10 mmol/liter of phosphate buffer pH 7.4, 5 mmol/liter EDTA. (*c*) 10 mmol/liter Tris-HCl buffer pH 7.8, 5 mmol/liter EDTA. (*d*) 10 mmol/liter Tris–HCl buffer pH 7.8. (*e*) 10 mmol/liter Tris-HCl buffer pH 7.8, 50 mmol/liter NaCl. The elution rate was 2 ml/h.

FIGURE 7 The influence of the ionic strength on the behavior of purified T5-receptor preparations on Bio-Gel A-50m columns (0.9 × 58.0 cm.) at 15°C in the presence of 0.3% Triton X-100. The amount of protein was determined by liquid scintillation counting of ^{14}C-labeled protein. In every run 0.032 mg of protein was applied in 0.05 ml of elution buffer. All runs were performed in the presence of 10 mmol/liter Tris-HCl (pH 7.8) and 0.02% of NaN_3 additionally to the additives given under (*a*) to (*e*). The triangle mark indicates the void volume. (*a*) Without Triton X-100. (*b*): 0.3% of Triton X-100. (*c*): 0.3% of Triton X-100, 10 mmol/liter NaCl. (*d*): 0.3% of Triton X-100; 50 mmol/liter NaCl. (*e*): 0.3% of Triton X-100, 300 mmol/liter NaCl. The elution rate was 2 ml/h.

resulting particles is heavily dependent on the ionic strength. Surprisingly, in spite of the enormous decrease of the particle size, no significant change in RU/mg of protein could be detected. Therefore the increase of the initial reaction rate $k_1 \times r_0 \times p_0$, caused by the addition of 0.3% Triton, which is given by the initial slope of the inactivation curve, is due only to an increase of k_1, and not to an increase of r_0. The influence of Triton on the rate constant k_1 increases slightly with pH (Fig. 8). The rate constant of the back reaction, k_2, is decreased to about one half in the whole pH range measured (Fig. 8).

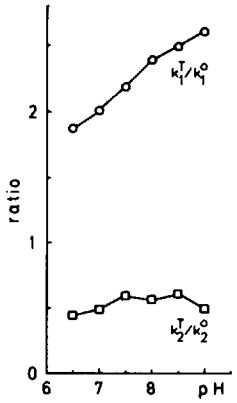

FIGURE 8 Ratios between the rate constants k_i^T, with 0.3% Triton X-100, and k_i^o without Triton as a function of the pH, at 35°C. The buffers were the same as described in Fig. 1.

The results of the kinetic experiments in the presence of 0.3% Triton X-100, which were performed under the same conditions as described in section III*d* with exactly the same receptor concentration and the same initial phage titer are shown by the symbols in Fig. 9. The much higher inactivation rate under Triton influence becomes obvious by comparing the experimental values in Fig. 3 and 9. In this case as well, the least-squares fits of Eq. 3a, calculated for each temperature separately, agree well with the experimental results (Fig. 9*a*). The rate constants thus obtained are plotted in Fig. 4. From Fig. 5 it follows that the temperature dependence of the k_i cannot be described by Eq. 6 throughout the whole temperature range. The dashed lines in the right part of Fig. 5 would be obtained, assuming that Eq. 6 is valid in the whole range, from 10° to 35°C. The correpsonding thermodynamic constants are given in Table I*b*. These enthalpies and entropies yield inactivation curves with poor agreement to the experimental data (Fig. 9*b*). The experimental values for $\ln(k_i/T)$ in the presence of Triton, shown in Fig. 5, suggest a separate treatment for two temperature ranges with a transition between 20° and 25°C. The values obtained from the exponential regression under this assumption (solid lines in Fig. 5), are given in Table I *c* and *d*. They result in well-fitting curves in Fig. 9 *c*.

To explain the discontinuity of the inactivation behavior caused by Triton, we examined the dependence of the size distribution of the receptor particles on temperature by gel chromatography on Bio-Gel A-1.5m (Fig. 10). The given molecular weights of the reference proteins are not corrected for bound Triton. On Bio-Gel A-1.5m, however, this binding should be negligible. Bovine serum albumin, binds about five molecules of Triton per molecule of protein, which would cause an error of about 3,000 mol wt (Sukow et al., 1980). Pure Triton X-100 micelles in this buffer system are eluted at an apparent molecular weight of 95,000 at 20°C and of 150,000 at 30°C (Paradies, 1980).

At 40°C we found a wide peak with a maximum at about 150,000 mol wt (Fig. 10 *c*). At 15°C this peak splits into three sharper ones, the first in the exclusion volume of the column, and the other two at ~300,000 and 80,000 mol wt, respectively (Fig. 10 *b*). This transition is reversible. Samples, kept at 40°C for 1 h, and cooled to the column temperature of 15°C before application, showed the same elution pattern as the 15°C run. As the percentage of

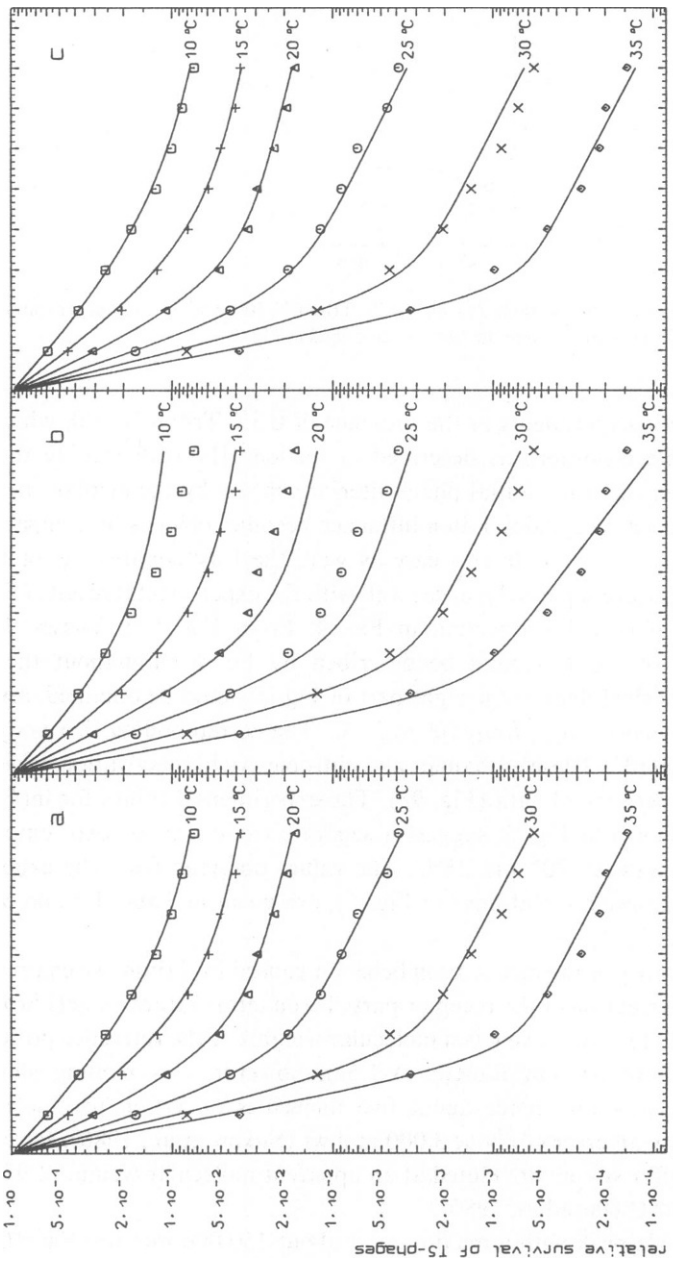

FIGURE 9 Inactivation of T5-phages by receptor in the presence of 0.3% of Triton X-100 as measured (symbols) and compared with curves calculated as follows: (*a*) from the rate constants k_1, k_2, and k_3 obtained by least-squares fitting for each temperature separately. (*b*): from the thermodynamic data $\Delta H_1^{\ddagger}$, $\Delta H_2^{\ddagger}$, $\Delta H_3^{\ddagger}$, $\Delta S_1^{\ddagger}$, $\Delta S_2^{\ddagger}$, and $\Delta S_3^{\ddagger}$, given in Table 1*b*, obtained by exponential regression of the k_i from (*a*). (*c*) as under (*b*) but for the temperature ranges from 10° to 20°C and from 25° to 35°C, separately. The data are given in Table 1*c* and *d*.

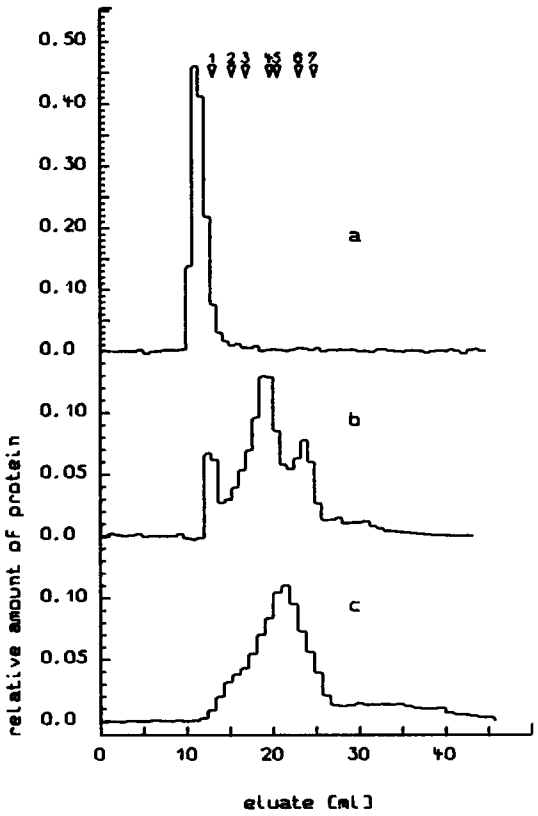

FIGURE 10 Behavior of purified T5-receptor preparations on Bio-Gel A-1.5m columns (0.9 × 58.0 cm) in dependence of the temperature and the presence of Triton X-100. The amount of protein was determined by liquid scintillation counting of ^{14}C-labeled protein. In every run 0.032 mg of protein in 0.05 ml of elution buffer was applied. All runs were performed in the presence of 10 mmol/liter Tris-HCl buffer (pH 7.8), 0.02% of NaN_3, 50 mmol/liter NaCl, and the additives given under (*a*) to (*c*). (*a*) Without Triton X-100, preincubation 1 h at 15°C, run temperature 15°C. (*b*) 0.3% of Triton X-100, preincubation 1 h at 15°C, run temperature 15°C. (*c*) 0.3% of Triton X-100, preincubation 1 h at 40°C, run temperature 40°C. The elution rate was 2 ml/h. The marks give the elution volumes of the following substances in the presence of 0.3% Triton: (1) Dextran blue (main peak). (2) Thyroglobulin 669,000 mol wt. (3) Ferritin 440,000 mol wt. (4) Catalase 232,000 mol wt. (5) Lactate dehydrogenase 140,000 mol wt. (6) Bovine serum albumin 67,000 mol wt. (7) Ovalbumin 43,000 mol wt.

protein in the void volume decreases with the applied protein concentration (not shown), Fig. 10 cannot reflect the real conditions in the reaction mixture for the kinetic studies, where the concentration of protein is 1,000 times lower.

If we consider the poor resolving power of the column, the molecular weight of the right peak in Fig. 10 *b* corresponds well to the molecular weight of the tonA gene product, the receptor protein for the T5-phage (Braun et al., 1973), which has been estimated by SDS polyacrylamide gel electrophoresis to be ~80,000 mol wt (Braun et al., 1976).

Each of the three peaks in the 15°C run showed almost the same specific activity in RU/mg protein and almost the same rate constant k_1, measured at 35°C in the presence of Triton

X-100, as the input to the column. Also the LPS to protein ratio of all three fractions did not change compared with the stock.

Dialyzing these fractions for 5 d against 0.02% NaN_3 at 10°C reduced the Triton concentration to less than a hundreth. Using these purified fractions in kinetic experiments without Triton (residual Triton concentration $<10^{-4}$ %) the same kinetic behavior was found as in experiments performed with receptor which was directly diluted from the receptor stock without Triton.

IV. DISCUSSION

(a) Influence of Heterogeneity of Phages on the Inactivation Process

The beginning of the reaction between T5-phages and its receptor, isolated from *E. coli* B, can be described by a one step, irreversible, biomolecular reaction model (Zarnitz and Weidel, 1963). However, as the reaction proceeds, the experimental values deviate considerably from the theoretical prediction. This discrepancy can be eliminated by introducing a reversible reaction step, as described in Eq. 1. The explicit solution for the corresponding system of differential equations, obtained under the restriction $r_0/p_0 >> 1$, is an expression which is the sum of two exponential terms (Eq. 3a). Under the assumption that the rate constants k_3 and k_2 are much smaller than $k_1 \times r_0$, which is the case for our experiments, we can simplify Eq. 3a to

$$p/p_0 = A_1 \cdot e^{-k_1 \cdot r_0 \cdot t} + A_2 \cdot e^{-k_3 \cdot t}. \tag{11}$$

For our k_i, the accuracy of the approximations of λ_1 and λ_2 is better than ± 1%. A similar equation for the inactivation can be derived, if two kinds of phages with different rate constants k' and k'' participate in a pseudomonomolecular, irreversible one-step reaction:

$$p/p_0 = (p_0'/p_0) \cdot e^{-k' \cdot r_0 \cdot t} + (p_0''/p_0) \cdot e^{-k'' \cdot r_0 \cdot t} \tag{12}$$

where p_0' and p_0'' are the initial titers of the two phage types, and p_0 and p denote the sums of the initial phage titers, and of the phage titers at time t, respectively. The exponent of the second term in Eq. 12 depends on the initial receptor concentration r_0, in contrast to Eq. 11 for our model.

This difference must become obvious if a set of curves which differ only in r_0 is calculated for the two models. Curves calculated for r_0 equal to $1/2 \times R_0$, $1 \times R_0$, $2 \times R_0$, and $4 \times R_0$ for both models are compared in Fig. 11. The receptor concentration R_0 is the one used in our experiments. The values of p_0'/p_0, p_0''/p_0, k', and k'' for the calculation of the curves in Fig. 11 *b* were chosen such that the curves for $1 \times R_0$ in Figs. 11 *a* and *b* become identical. The resulting ratio between the two kinds of phages p_0'/p_0'' would be about 1/1,000. Experimental results already published (Zarybnicky et al., 1980) support our model.

(b) The Inactivation Process in View of the Transition-State Theory

The transition-state theory allows for separation of the magnitude and temperature dependence of a rate constant in terms of the energy it takes to break the necessary bonds ($\Delta H^{\ddagger}$) and the change in 'disorder' of the reactants ($\Delta S^{\ddagger}$). By comparing the collision theory with the transition-state theory the term with $\Delta S^{\ddagger}$ in Eq. 6 can be interpreted as the probability of the

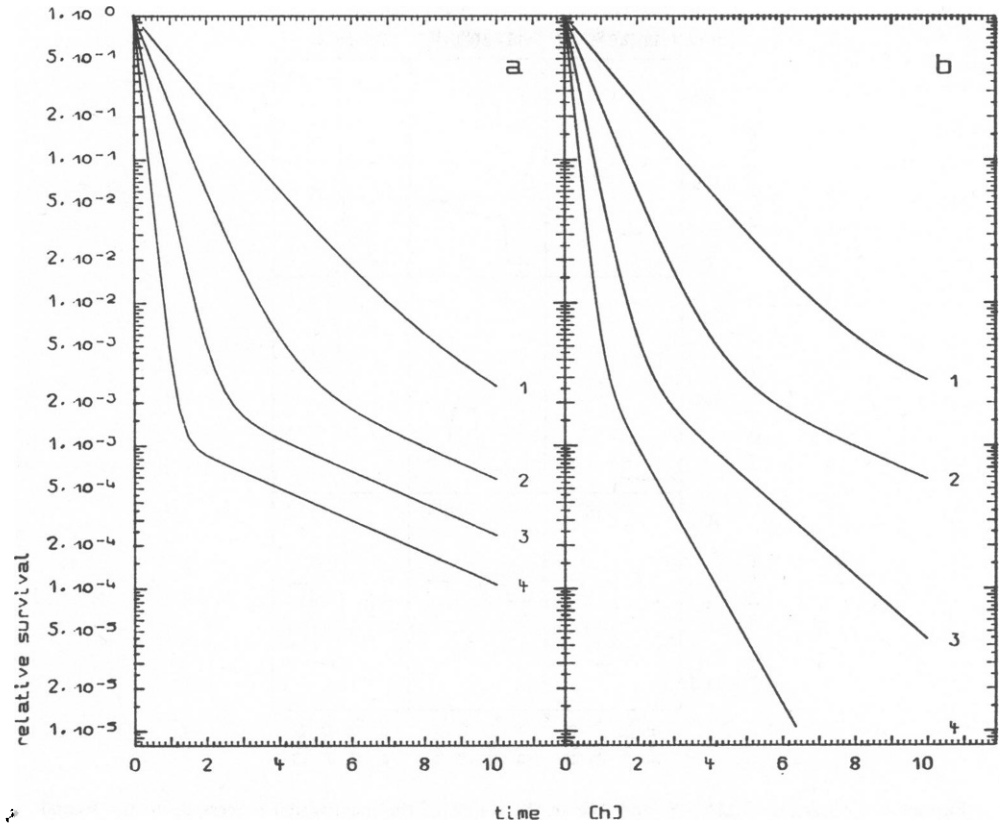

FIGURE 11 (*a*) Comparison of theoretical curves calculated for our model, according to Eq. 3a, and (*b*) for the assumption of a heterogeneity in the phage population, according to Eq. 12, in dependence on the initial receptor concentration. The curves 1, 2, 3, and 4 were calculated for the receptor concentrations r_o equal to $\frac{1}{2} \times R_o$, $1 \times R_o$, $2 \times R_o$, and $4 \times R_o$, respectively. The concentration, R_o, was the one used in all experiments. (*a*) shows curves, calculated from the k_i of our results for 35°C in the absence of Triton. The variables for (*b*) were chosen such that the curves 2 in (*a*) and (*b*) become identical.

formation of the activated complex. It is obvious that the probability increases with increasing $\Delta S^{\ddagger}$.

The thermodynamic data ΔH, ΔS, and ΔG for the first two reaction steps, up to the second activated complex, are depicted schematically in Fig. 12. The values were taken from Table I*a*, *c*, and *d*. For the incubation without Triton X-100 it is remarkable that the entropy of activation for the first reaction step, $\Delta S_1^{\ddagger}$, is near zero, which makes the Gibbs free energy of activation, $\Delta G_1^{\ddagger}$, practically independent of temperature. The enthalpy of activation, $\Delta H_1^{\ddagger}$, is relatively low. This means that only weak interactions are involved. The corresponding energy of activation E_A (40 kJ/mol) differs only slightly from that found by Zarnitz and Weidel (1963) (44.4 kJ/mol) for their irreversible, bimolecular, one-step approximation.

Proceeding from the activated complex $\langle PR \rangle^{\ddagger}$ to $\langle PR \rangle$, a further increase in the enthalpy and a large increase in the entropy occurs. This makes the first reaction step entropy driven, with a change in Gibbs free energy $\Delta G_{1,2} = -74.2$ kJ/mol (35°C), yielding a value of 3.8 ×

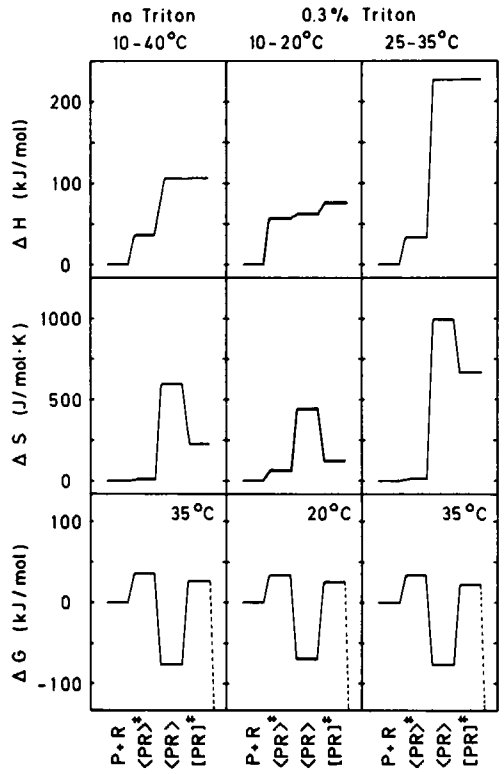

FIGURE 12 Changes of ΔH, ΔS, and ΔG, in the course of the inactivation process up to the second transition-state. (For symbols see Eq. 1).

10^{12} liter/mol for the equilibrium constant $K_{1,2}$. For our receptor concentration this would result in the equilibrium ratio $\langle pr \rangle / p = 1.93 \times 10^2$, which could only be reached in the hypothetical case that k_3 is equal to zero. The maximal concentration of $\langle PR \rangle$, calculated from our value of k_3 at 35°C, is found at 1.45 h with 68% of p_0.

The change in free energy, $\Delta G_3^{\ddagger}$, needed to reach the second activated complex $[PR]^{\ddagger}$, is about three times larger than $\Delta G_1^{\ddagger}$. In contrast to the building of $\langle PR \rangle^{\ddagger}$, this transition seems to be governed by structural changes of the complex to higher order, resulting in a decrease in entropy, although the enthalpy does not change. Therefore the rate constant k_3 is only linearly dependent on the absolute temperature. As the remainder of the inactivation reaction is irreversible, ΔG for the whole reaction must be large and negative.

(c) Influence of Triton X-100

Triton X-100 is often used for the solubilization of membrane proteins. In former kinetic experiments we found an increase of the initial inactivation rate due to the addition of Triton. Therefore we studied the temperature dependence of the inactivation of T5-phages by receptors in the presence of Triton, as well. The thermodynamic behavior, however, was much more complex than in the absence of Triton. The reaction cannot be described by one set of thermodynamic data (Table I *b* and Fig. 9 *b*). At least two sets of thermodynamic data are needed for a satisfying description (Table I *c* and *d*). The division of the measuring range into

the two temperature ranges, 10° to 20°C and 25° to 35°C, permits a good theoretical fitting of the experimental curves (Fig. 9 *c*). Comparing the Gibbs free energy diagrams in Fig. 12, a considerable similarity in all steps is evident. This means that no essential structural changes in the binding sites of both reactants, the receptor protein and the phage tail, occur because of the influence of Triton. The changes of enthalpy and entropy in the presence of Triton are qualitatively comparable to those in the absence of Triton.

Between 25° and 35°C the addition of 0.3% Triton X-100 to the incubation buffer causes an increase of the relative initial inactivation rate $k_1 \times r_0$ by a factor between 2.13 and 2.19 (Fig. 13). Electron micrographs in the absence of Trition X-100 show that 95% of the large, Bio-Gel A-50 m purified receptor particle are able to inactivate only one phage. This is true, even if the phages are in large excess over the receptors, and after long incubation times. The addition of Triton X-100 does not significantly influence the specific activity of the receptor preparation, measured as receptor units per milligram of protein as described in Materials and Methods (II *e*). The behavior of the receptors on Bio-Gel A-50m and on Bio-Gel A-1. 5m columns show that the macroparticles are split by Triton to about a hundredth of their original size (Figs. 7 and 10). The split particles are heterogeneous in size and may contain other outer membrane proteins. Considering the fact that the specific activity is not enhanced by the splitting of the receptor particles, the increase found in the initial reaction rate can be interpreted as a true increase in the rate constant k_1.

Two explanations for the interaction of the phage and the split receptors are possible. Either, (*a*) from one large particle only one small active particle is set free, or (*b*) the splitting results in several active subparticles. In the latter case, several receptor subparticles must be bound to the phage tail in order to inactivate the phage. Under this assumption our model for the inactivation process should be extended by several steps before the complex ⟨PR⟩ can be built. These reaction steps may, however, be very fast and perhaps cooperative so that they are immeasurable under our experimental conditions.

The diameter of a receptor particle without Triton and for a T5-phage head can be approximated from electron micrographs to be ~30 and 65 nm, respectively. Because the

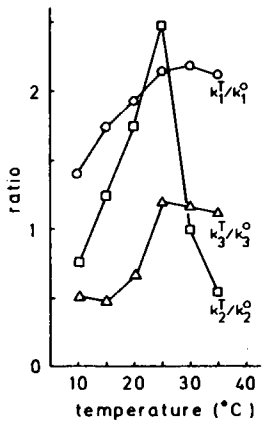

FIGURE 13 Temperature dependence of the ratios of the rate constants k_i^T (with 0.3% of Triton X-100) to k_i^0 (without Triton X-100).

phage is asymmetrical, we assume a Stokes diameter of ~70 nm. The Bio-Gel A-1.5 m run of the receptor in the presence of Triton at 40°C (Fig. 10 *c*) shows an average elution volume corresponding to a Stokes diameter of ~9 nm.

The term q_i in Eq. 8 can be interpreted in view of the collision theory as a product of the collision frequency and a probability (steric) factor. Assuming that the probability factor did not change, the increase of the rate constant k_1 for constant $\Delta H_1^{\ddagger}$ (see Table I), due to the higher mobility of the Triton-treated particles, can be calculated by the following equation:

$$\frac{k_1^T}{k_1^0} = \frac{(D_A^T + D_B^T) \cdot (r_A^T + r_B^T)}{(D_A^0 + D_B^0) \cdot (r_A^0 + r_B^0)} \tag{13}$$

where D and r are the diffusion coefficients and the Stokes radii for the reacting components A, (index A), and B, (index B), with (upper index T) and without (upper index 0) Triton X-100, respectively. Eq. 13 is derived from the formula for the encounter rate constants of diffusion-controlled reactions, given by Gutfreund (1972). Inasmuch as the viscosity, η, of the Triton buffer does not significantly differ (<0.5%) from that of the Triton-free buffer, the introduction of $D = k \times T/(6 \times \pi \times \eta \times r)$ where k is the Boltzmann constant and T is the absolute temperature for the diffusion coefficient simplifies Eq. 13 to a form that only depends on the Stokes radii:

$$\frac{k_1^T}{k_1^0} = \frac{r_A^0 \cdot r_B^0}{r_A^T \cdot r_B^T} \cdot \left(\frac{r_A^T + r_B^T}{r_A^0 + r_B^0}\right)^2. \tag{14}$$

Using the values above, a ratio for k_1^T/k_1^0 of 2.08 results. If we calculate the Stokes radii from the diffusion coefficients for the T5-receptor, given by Weidel et al. (1954), for the T5-phage, given by Dubin et al. (1970), and for catalase (marker protein in Fig. 11), given by Tanford (1961), we get a ratio of 2.45. Both values agree well with the experimental values given above.

This means that the increase of the rate constant k_1 in the upper temperature range, caused by the addition of Triton, is only due to the higher mobility of the split particles. In terms of the transition-state theory this must result in a corresponding small increase of the entropy, which determines the probability of the formation of the activated complex $\langle PR \rangle^{\ddagger}$, whereas $\Delta H_1^{\ddagger}$ should remain constant. Our data (Table I *a* and *d*) coincide very well with this hypothesis. This result and the fact that we found no visible receptor particles at phage tails on electron micrographs in the presence of Triton support the assumption that only a small fraction of the originally large particles must be bound to the phage for its inactivation.

Proceeding from the activated state $\langle PR \rangle^{\ddagger}$ to the intermediate complex $\langle PR \rangle$ in the upper temperature range, the high increase of the enthalpy is compensated by a comparably high increase of the entropy, yielding nearly the same $\Delta G_2^{\ddagger}$ as without Triton (see Fig. 12). The high increase of enthalpy $\Delta H_2^{\ddagger}$ results in a much higher temperature dependence of k_2 (Fig. 4). The decrease of k_2 together with the increase of k_1 with temperature results in a faster decrease of the reversibility of the first reaction step than in experiments without Triton. The equilibrium constant $K_{1,2}$ (liter/mol) in the presence of Triton rises from 162 at 25°C to 3,368 at 35°C, compared with an increase from 181 at 25°C to only 770 at 35°C in the absence of Triton. The rate constant of the second reaction step, k_3, seems, at least in the upper temperature range, to be independent of the influence of the detergent.

On account of the far-reaching similar behavior of the reaction under both experimental conditions above 25°C, we can conclude that the detergent does not significantly change the conformation of the receptor protein. This could be explained by the incorporation of the receptor protein into a Triton micelle, which simulates a membranelike environment. Under the assumption that in the 40°C Bio-Gel A-1.5m run (Fig. 10*c*) all protein molecules are separated from each other, such mixed micelles would fit the elution volume of the receptor activity.

There are two reasons that the reliability of the results is restricted for the lower temperature range in the presence of Triton. First, the receptor material is highly heterogeneous in size as can be seen from Figure 10*b*. Second, the inactivation curves obtained for the experimental parameters depend only weakly on k_2 and k_3, yielding a large uncertainty in the mathematical approximation of these rate constants. To show the grade of reliability of the thermodynamic data for the lower temperature range we calculated the values of the error, E (see Appendix), for theoretical curves, obtained from thermodynamic constants in the vicinity of our result, given in Table I *c*. Table II gives the ranges of thermodynamic constants which result in curves with E between 0.068 and 0.069. This error corresponds to a deviation of the single measured value from the theoretical curve of $< \pm$ 12%, which is a usual accuracy for the plating of phages. No set of curves with an error of <0.068 could be found. Outside the limits given in Table II, the value of E increases steeply. As expected, the width of the ranges for $\Delta H_2^{\ddagger}$, $\Delta H_3^{\ddagger}$, $\Delta S_2^{\ddagger}$, and $\Delta S_3^{\ddagger}$ are about ten times larger than those for $\Delta H_1^{\ddagger}$ and $\Delta S_1^{\ddagger}$, respectively. From Table II, it follows that $\Delta H_1^{\ddagger}$ and $\Delta S_1^{\ddagger}$ between 10° and 20°C differ significantly from the values between 25° and 35°C. In the lower temperature range, $\Delta H_1^{\ddagger}$ is ~20 kJ/mol and $\Delta S_1^{\ddagger}$ is ~70 J/(mol × K) higher. The incomplete splitting of the Triton-treated particles below 25°C seems to make necessary a greater $\Delta H_1^{\ddagger}$ to force the receptor protein into a conformation capable to bind the phage. This structural change might be accompanied by a higher increase of the entropy as in the upper temperature range.

The influence of Triton X-100 on the second reaction step, from ⟨PR⟩ to [PR], is small in all cases. For the lower temperature range, the value near zero found for $\Delta H_3^{\ddagger}$ for the reaction without Triton and between 25° and 35°C for the reaction with Triton, is included in the

TABLE II

RANGES OF THE THERMODYNAMIC CONSTANTS OF T5-PHAGE ADSORPTION TO ISOLATED T5-RECEPTORS IN PRESENCE OF TRITON, IN THE TEMPERATURE RANGE FROM 10°C TO 20°C

		Minimum	Maximum
$\Delta H_1^{\ddagger}$	(kJ/mol)	57.1	59.5
$\Delta H_2^{\ddagger}$	(kJ/mol)	−22.0	+7.7
$\Delta H_{1,2}$	(kJ/mol)	51.4	77.5
$\Delta H_3^{\ddagger}$	(kJ/mol)	−0.6	15.9
$\Delta S_1^{\ddagger}$	[J/(mol × K)]	75.8	89.7
$\Delta S_2^{\ddagger}$	[J/(mol × K)]	−411.5	−310.8
$\Delta S_{1,2}$	[J/(mol × K)]	398.4	487.3
$\Delta S_3^{\ddagger}$	[J/(mol × K)]	−336.9	−145.3

The given limits include all thermodynamic constants found with an error, E (see Appendix), of <0.069. This error corresponds to a value of E calculated for usual experimental errors of phage-titer estimation.

experimental standard error in Table I *c*, as well as in the limits in Table II. Only the entropy change, $\Delta S_3^{\ddagger}$, below 25°C and in presence of Triton, was found to be slightly smaller. This causes a decrease of the rate constant k_3 (Fig. 4). The insensitivity to Triton suggests that this irreversible reaction step occurs in the phage tail. It should be involved in conformational changes in the phage tail, preparing its opening for the ejection of the DNA.

APPENDIX

Numerical Methods

Inasmuch as the receptor in all our experiments is in large excess over phages, we could use the explicit solution (Eq. 3a) for the system of nonlinear differential equations (Eqs. 2a–d). The rate constants k_i of this solution were fitted to the experimental results by the method of least squares for each temperature, separately. This was done on a TR440 computer at the *Zentrum für Datenverarbeitung der Universität Tübingen*. The least-squares fit was performed by a Fortran bibliothek subroutine[2] using a finite difference Levenberg-Marquardt algorithm (Brown and Dennis, 1972). Since the experimental values vary over a range of $\sim 10^4$, we used for the deviation, D, the relation

$$D = \frac{X_{\text{exp}} - X_{\text{calc}}}{X_{\text{exp}} + X_{\text{calc}}} \tag{15}$$

for the least-squares fits. The subroutine minimizes the sum of the squares of these deviations by varying the rate constants k_i.

The calculated results were controlled graphically by comparing the plots of measured and calculated values and numerically by the error, E:

$$E = \sqrt{\frac{1}{n - m}} \cdot \sum_{i=1}^{n} D_i^2 \tag{16}$$

where n and m are the numbers of measured values and of variables, respectively. If we apply the transformations

$$Z = \ln \frac{k_i}{T} \quad \text{and} \quad X = \frac{1}{R \cdot T}$$

to Eq. 8, we obtain the linear regression function $Z = a + b \cdot x$, where

$$a = \ln \frac{k}{h} + \frac{\Delta S_i^{\ddagger}}{R} \quad \text{and} \quad b = -\Delta H_i^{\ddagger}.$$

Applying the formulae for regression coefficients a and b derived from general theory of least-squares method (Rektorys, 1969), we obtain for enthalpy and entropy of activation the following relations:

$$\Delta H_i^{\ddagger} = -R \cdot \frac{\sum_{n=1}^{n} \left(\frac{1}{T_n} \cdot \ln \frac{k_{i,n}}{T_n}\right) - \frac{1}{n} \cdot \left(\sum_{n=1}^{n} \frac{1}{T_n}\right) \cdot \left(\sum_{n=1}^{n} \ln \frac{k_{i,n}}{T_n}\right)}{\sum_{n=1}^{n} \left(\frac{1}{T_n}\right)^2 - \frac{1}{n}\left(\sum_{n=1}^{n} \frac{1}{T_n}\right)^2} \tag{17}$$

[2]Subroutine ZXSSQ, International Mathematical And Statistical Libraries, Inc., Houston, Texas 77036, U. S. A.

$$\Delta S_i^{\ddagger} = R \cdot \left[\frac{1}{n}\left(\sum_{n-1}^{n} \ln \frac{k_{i,n}}{T_n} + \frac{1}{R} \cdot \Delta H_i^{\ddagger} \cdot \sum_{n-1}^{n} \frac{1}{T_n}\right) - \ln \frac{k}{h}\right] \tag{18}$$

The corresponding estimates of standard errors are

$$S_{H_i} = R \cdot \sqrt{\frac{(1 - r_i^2) \cdot \left[\sum_{n-1}^{n} \left(\ln \frac{k_{i,n}}{T_n}\right)^2 - \frac{1}{n} \cdot \left(\sum_{n-1}^{n} \ln \frac{k_{i,n}}{T_n}\right)^2\right]}{(n-2) \cdot \left[\sum_{n-1}^{n} \left(\frac{1}{T_n}\right)^2 - \frac{1}{n} \cdot \left(\sum_{n-1}^{n} \frac{1}{T_n}\right)^2\right]}} \tag{19}$$

$$S_{S_i} = R \cdot \sqrt{\frac{(1 - r_i^2) \cdot \sum_{n-1}^{n} \left(\frac{1}{T_n}\right)^2}{n \cdot (n-2) \cdot \left[\sum_{n-1}^{n} \left(\frac{1}{T_n}\right)^2 - \frac{1}{n}\left(\sum_{n-1}^{n} \frac{1}{T_n}\right)^2\right]}} \tag{20}$$

where r_i is the Pearson Bravais correlation coefficient defined as:

$$r_i = \frac{\sum_{n-1}^{n} \left(\frac{1}{T_n} \ln \frac{k_{i,n}}{T_n}\right) - \frac{1}{n} \cdot \left(\sum_{n-1}^{n} \frac{1}{T_n}\right) \cdot \left(\sum_{n-1}^{n} \ln \frac{k_{i,n}}{T_n}\right)}{\sqrt{\sum_{n-1}^{n} \left(\frac{1}{T_n}\right)^2 - \frac{1}{n} \cdot \left(\sum_{n-1}^{n} \frac{1}{T_n}\right)^2} \cdot \sqrt{\sum_{n-1}^{n} \left(\ln \frac{k_{i,n}}{T_n}\right)^2 - \frac{1}{n} \cdot \left(\sum_{n-1}^{n} \ln \frac{k_{i,n}}{T_n}\right)^2}}. \tag{21}$$

Replacing $k_{i,n}/T_n$ in formulae 17–21 with $K_{1,2}$ we obtain the corresponding relations for $\Delta H_{1,2}$, $\Delta S_{1,2}$, $S_{H_{1,2}}$ and $S_{S_{1,2}}$.

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