Quantification and Localisation of SH-Groups in Human Blood Serum Proteins

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The thiol groups of human blood serum proteins were determined after 24 hours interaction with dithionitrobenzoic-acid (DTNB) to an average of 538 ± 60 μmol/l serum. After treatment of the serum with [^{35}S]DTNB, autoradiograms of the protein elpherograms revealed two main peaks: The first with 63% of total activity, in the albumin region, corresponding to 0.60 SH/mol, the second with 23% of total activity, in the γ-globulin range, corresponding to 2.2 SH/mol. After 30 minutes incubation with DTNB, or with *p*-chloromercuribenzoate (CMB), in freshly prepared pools of IgG only 0.2 SH/mol were found which is the expected value already known from the literature.

Autoradiograms taken from serum protein elpherograms after interaction with [^{14}C]CMB only show the main SH-peak in the albumin range. Thus ist is concluded that the SH-peak in the γ-globulin region after 24 hours incubation with [^{35}S]DTNB is due to one highly labile S-S-bond which easily undergoes a disulfide exchange with DTNB.

Blood serum freshly taken from 50 healthy male probands (20 to 45 years old) is diluted 1:10 with 0.1 M phosphate buffer pH 7.0 and subsequently mixed with the tenfold volume of phosphate buffer containing 1×10^{-2} M 5.5′-dithio-bis-nitrobenzoic-acid (DTNB).

After 24 hours reaction time at 4 ° in the dark the absorbance of the samples were measured at 412 nm. From the values corrected for blank absorbance of DTNB and serum, respectively, it was computed that human serum contains an average of 538 ± 60 μmol protein SH-groups per liter.

Similarly, the serum samples were treated with ^{35}S-labeled DTNB and separated electrophoretically on cellulose acetate strips (buffer pH 8.4, 110 V, 2 h). The autoradiograms prepared from the strips by 4 to 8 weeks exposure to an X-ray film exhibited a distinct main band in the albium region and another weaker one in the γ-globulin region. The quantitative densitometry revealed that (in the average) 63% of total radioactivity was present in the albumin peak, 23% in the γ-globulin peak and 14% scattered rather uncharacteristically in the region of α- and β-globulin. Based on these values and the (simultaneously estimated) content of albumin, γ-globulin and total SH-groups in the serum it was calculated that albumin (mole weight 69,000) has an SH content of 0.60 mol per mole and γ-globulin (mole weight 160,000) 2.2 mol per mol.

The value found for albumin is in excellent agreement with the already known SH content of isolated serum albumin and may thus be used as an internal standard for the method.

The SH content found for γ-globulin, however, appears surprisingly high, as an SH content of only 0.2 reactive SH/mol IgG which comprises the major part of the γ-globulin fraction, is reported in the literature [1].

This value was also confirmed by own experiments with freshly prepared pools of IgG. When these samples were incubated for 30 min with DTNB the developed yellow colour corresponded to 0.2 mol SH/mol IgG. Additional experiments using *p*-chloromercuribenzoate (CMB) as SH reagent yielded similar values. Furthermore, autoradiograms of electrophoretically separated serum samples labeled with [^{14}C]CMB instead of [^{35}S]-DTNB showed only one mayor peak (70% of total radioactivity) in the albumin region, but no distinct peak in the γ-globulin area.

These different results in respect to the SH content of the γ-globulin fraction suggest, that the γ-globulins contain in addition to 0.2 mol of reactive SH/mol also one highly labile disulfide bond, which reacts during long time incubation with DTNB in a disulfide exchange reaction [2].

$$\text{Prot} - \text{SH} + \text{XSSX} \qquad \text{Prot} - \text{SX} + \text{HSX}$$

$$\text{Prot} \begin{matrix} \text{S} \\ \text{S} \end{matrix} + \text{XSSX} \qquad \text{Prot} \begin{matrix} \text{SSX} \\ \text{SSX} \end{matrix}$$

The experiments are being continued for further clarification of the suggested secondary reaction of DTNB and in collaboration with W. List * and coworkers for investigations of pathalogical serum samples.

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Host Factors Involved in the Growth of Microvirid Phage α 3

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Host factors involved in the growth of microvirid phage α3 were determined using various replication mutants of *Escherichia coli*. The viral multiplication was dependent on functional products of *dnaE*, *dnaF(nrdA)*, *dnaG*, and *dnaZ* genes. Host functions directed by *dnaA*, so-called *dnaH*, *dnaI*, and *dnaP* genes were dispensable for the viral growth. In contrast with φX174 and G4, α3 would grow sufficiently in *dnaB* and *dnaC(D)* mutants. The viral growth was not significantly affected by host *polA^{ts}*, *seg*, and *groP*C mutations.

Introduction

Recently, considerable diversities have been detected among microvirid (isometric) phages, concerning host factor dependence, immunological relationship, and host range. Thus φX174 members (*e. g.* φA, S13, G6) infect *E. coli* C and require host *dnaB* and *dnaC(D)* functions for their growth [1–3]. These two host functions are essential for immunologically unrelated phage G4 [4]. Moreover, G13 and G14, which can infect *E. coli* B as well, rely on *dnaB* and *dnaC(D)* activities [5]. On the other hand, neither *dnaB* nor *dnaC(D)* gene product is required for multiplication of K12-specific group (including φK and St-1) [6] which is immunologically remote from φX174. Assignment of host factors required for these phages is important for elucidation of mechanisms of viral replication and evolution. This report describes the host factor reliance of α3 [7] which is infective to *E. coli* C and B and immunologically somewhat related to St-1 but not to φX174.

Materials and Methods

E. coli C-N27 *polA4113* [8] was obtained from Dr. T. Okazaki. The sources of other *E. coli* strains used were previously described [1–6, 9–12]. Unless otherwise specified, bacteria were grown in a nutrient broth, at 30 °C, with shaking. Phage α3, originally provided by Dr. D. E. Bradley, was propagated on *E. coli* C and partially purified by differential centrifugation. Single-stranded viral DNA (SS) and double-stranded replicative-form DNA (RF) of α3 were prepared as previously described [13]. Infection experiments with α3 phage were performed as described for φK[6]. Bacterial strains resistant to α3 were transfected with α3 SS or RF, after Ca^{2+}-treatment [1]. The free phage titer was determined using *E. coli* C as the indicator.

Results and Discussion

Effects of various host mutations on the growth of α3 are summarized in Table I. Like other microvirid phages, α3 could grow sufficiently in *dnaA* cells. Moreover, the viral growth proceeded normally in *dnaB* and *dnaC(D)* mutants, at 43 °C. On the other hand, multiplication of φX174, φA,

Table I. Growth of α3 in replication mutants of *E. coli*.

Strain	Phage yield		
	43 °	33 °	43 °/33 °
C *dna*$^+$	(2.4×10^6) [a]	2.3×10^6	1.0
C2307 *dnaA*	(4.2×10^7) [a]	3.2×10^7	1.3
LD312 *dnaB*	1.9×10^7	6.6×10^6	2.9
LD332 *dnaC(D)*	(1.7×10^6) [a]	1.6×10^6	1.1
LD301 *dnaE*	1.0×10^4	2.5×10^5	4.0×10^{-2}
JG42 *dnaF*	(1.1×10^5) [b]	(4.7×10^6) [b]	2.3×10^{-2}
C2309 *dnaG*	8.0×10^3	7.0×10^5	1.1×10^{-2}
HF4704S *dnaH*	(7.3×10^5) [a]	7.2×10^5	1.0
WM301-208 *dnaI*	(1.9×10^7) [a]	1.3×10^7	1.5
KM107 *dnaP*	(7.7×10^6) [c]	9.1×10^6	8.5×10^{-1}
AX727 *dnaZ*	(1.6×10^3) [b]	(1.4×10^6) [b]	1.1×10^{-3}
C727 *dnaZ*	1.5×10^4	2.2×10^6	6.8×10^{-3}
C-N27 *polA 4113*	6.9×10^6	2.4×10^7	2.9×10^{-1}
BT4113 *polA^{ts}*	(3.3×10^5) [b]	(5.2×10^5) [b]	4.4×10^{-1}
KS268 *ligts7*	(1.8×10^4) [b]	(3.0×10^6) [b]	6.0×10^{-3}
BW2001 *xth-11*	(2.0×10^5) [b]	(2.6×10^6) [b]	7.7×10^{-2}
PB213 *seg*$^+$	(3.4×10^3) [b]	(6.6×10^3) [b]	5.2×10^{-1}
PB1022 *seg-2*	(1.1×10^3) [b]	(3.8×10^3) [b]	2.9×10^{-1}
PB1022 *seg-2*	(2.3×10^5) [d]	(3.7×10^4) [d]	6.2
C600 *gro*$^+$	(3.4×10^4) [b] *	(4.1×10^4) [b]	8.3×10^{-1}
MF634 *groP*C259	(1.3×10^4) [b] *	(2.6×10^4) [b]	5.0×10^{-1}
C600 *groP*C756	(9.9×10^3) [b] *	(7.1×10^4) [b]	1.4×10^{-1}
C600 *groP*C756	(1.9×10^5) [d] *	(5.3×10^5) [d]	3.6×10^{-1}

[a] Cells were grown for 60 min at 43 °C before infection.
[b] Phage yield was determined by transfection of SS DNA to the Ca^{2+}-treated bacteria.
[c] Cells were grown for 100 min at 43 °C prior to infection.
[d] Phage yield was determined by transfection of RF DNA to the Ca^{2+}-treated cells.
* Incubation temperature was 43.5 °C.

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S13, and G6 was clearly restricted in these hosts, at the high temperature. In *dnaE*, *dnaF(nrdA)*, and *dnaG* mutants, growth of $\alpha 3$ was thermosensitive, indicating involvement of host DNA polymerase III, ribonucleotide reductase, and primase in the viral replication. Yield of $\alpha 3$ was not reduced at 43 °C in HF4704S "*dnaH*" which was recently shown to carry double mutations in *dnaA* gene and in some step for utilization of exogenous thymine [14]. (Among enzymes participated in thymine utilization, thymidine phosphorylase and purine nucleoside phosphorylase were not particularly defective in HF4704S strain.) At 43 °C, replication of $\alpha 3$ was not significantly affected in strains WM301-208 *dnaI* and KM107 *dnaP*. Furthermore, growth of φX174 was not thermosensitive in the *dnaP* strain (unpublished observation). In contrast, multiplication of $\alpha 3$ was distinctly thermosensitive in AX727 *dnaZ* mutant transfected with $\alpha 3$ SS, as well as in C727 *dnaZ* cells infected with intact $\alpha 3$ phage. Functional product of *dnaZ* gene is essential for all microvirid phages thus far tested. It must be noted here that host functions directed by *dnaE*, *dnaF(nrdA)*, *dnaG*, and *dnaZ* genes are essential for λ phage as well.

Although multiplication of $\alpha 3$ was only slightly affected by $polA^{ts}$ mutation, the phage yield was markedly reduced at 43 °C in strain KS268 *ligts7*, indicating participation of host DNA ligase in the viral growth. In strain BW2001 *xth-11*, growth of $\alpha 3$ was considerably reduced at 43 °C. However, whether exonuclease III, product of the *xth* gene, is directly involved in $\alpha 3$ replication process (*e. g.* removal of primer RNA) or not is presently unknown. Replication of $\alpha 3$ was not significantly affected at 43 °C in PB1022 *seg-2* cells, whereas growth of λ phage was, as reported by Jamieson and Bergquist [15], abortive in this mutant at 42 °C – 43 °C (data not shown). In contrast with λ, $\alpha 3$ could grow normally in *groP*C mutants, at 37 °C. Yield of $\alpha 3$ in strains MF634 *groP*C259 and C600 *groP*C756 was not particularly reduced even at 43.5 °C, as compared with the yield at 33 °C. Unlike λ phage, $\alpha 3$ requires host functions specified by *rep* gene (data not shown).

Host range of $\alpha 3$ is similar to that of G13 and G14: these phages can infect *E. coli* BB and BB5 but not to BB2, BB1 BB20, BB7, BB9, BB4 and BB12 (unpublished observation). Host factor requirement of $\alpha 3$ nevertheless differs from that of G13 and G14, and closely resembles that of K12-specific phages St-1 and φK [6]. Moreover, φKh-1 (a host range mutant of φK) [2] can infect *E. coli* K12, C, BB, BB5, BB1, and BB7, whereas host factor reliance of this phage is essentially similar to St-1, φK, and $\alpha 3$. These results are consistent with the fact that $\alpha 3$ is immunologically related to St-1 group [7] but quite different G13 and G14.

Dispensability of *dnaB* and *dnaC(D)* functions for $\alpha 3$ predicts that this phage, like G4 [16], may have a unique origin of synthesis of complementary (minus) DNA strand. Furthermore, in synthesis of viral (plus) DNA strand, $\alpha 3$ system is by far simpler than G4 which, like φX174, requires *dnaB* and *dnaC(D)* genes products for this reaction [4]. Determination of nucleotide sequence of the origin of plus strand synthesis is essential for characterization of this unique replication system.

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