CONVERSION OF NON-COVALENT INTERACTIONS IN NUCLEOPROTEINS INTO COVALENT BONDS: BISULFIDE-INDUCED FORMATION OF POLYNUCLEOTIDE–PROTEIN CROSSLINKS IN MS2 BACTERIOPHAGE VIRIONS*

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1. Introduction

The action of nucleophilic reagents like hydroxylamines or bisulfite upon DNA phages gives rise to protein–polynucleotide crosslinks [1–3] which are formed presumably due to conversion of particular non-covalent interactions between non-helical cytosine residues and phage protein amino acid residues into covalent bonds [1–6].

We now report the formation of a limited number of covalent polynucleotide–protein crosslinks in an spherical RNA bacteriophage MS2 induced by treatment with bisulfite. Both maturation and coat proteins are involved in this crosslinkage.

These data indicated the presence of some specific non-covalent interactions between RNA and proteins inside the virion of spherical RNA bacteriophages.

2. Materials and methods

Bacteriophage MS2 was grown by a routine method [7] and purified by polyethylene glycol precipitation [8] followed by centrifugation in a preformed gradient of CsCl for 2 hr at 35 000 rpm [9]. The resulting phage preparation (5×10^{14} pfu, 200 units of A_{260} per ml) was stored at 4°C. The solution of *N*-[^{14}C]-acetoxysuccinimide (0.1 M in absolute dioxane, 5 μCi/μmole) was obtained as described earlier [10].

The phage suspension was mixed with an equal volume of $NaHSO_3$ solution containing 2×10^{-2} M $MgCl_2$. The final $NaHSO_3$ concentrations and pH are specified in figures.

During the modification, it was necessary to avoid prolonged contact of the reaction mixtures with air because side reactions of bisulfite with oxygen [11] made the results poorly reproducible. The complication was overcome by performing the modification in capillary tubes.

To estimate the amount of protein crosslinked with RNA, 6 μl aliquots of reaction mixtures were added to 50 μl 0.05–0.17 M K_2CO_3 to bring the pH value to 8.5 and *N*-[^{14}C]acetoxysuccinimide added to label the proteins according to ref. [10]. To remove the protein which was non-bound covalently with RNA, phenol deproteinization on a thin-layer DEAE-cellulose plate was performed as described in the accompanying paper [12]. The results are presented in fig. 1.

To isolate the RNA–protein crosslinkage product, a 100 μl aliquot of the reaction mixture was brought to pH 8.5 by adding 30 μl 0.5 M K_2CO_3 and subjected to gel-filtration on a Sephadex G-25 column (7×2 cm) in 0.5 M NaCl (flow rate 1 ml/min) to remove bisulfite. The phages were precipitated with polyethyleneglycol, and the precipitate suspended in 7 M urea–0.5 M NaCl–1% SDS. The suspension was heated for 30 min at 60°C and cooled to 20°C; 0.05 ml 1 M phosphate buffer, pH 8.0, was added followed by 0.1 ml of *N*-[^{14}C]acetoxysuccinimide solution. In 30 min of incubation at 20°C, the mix-

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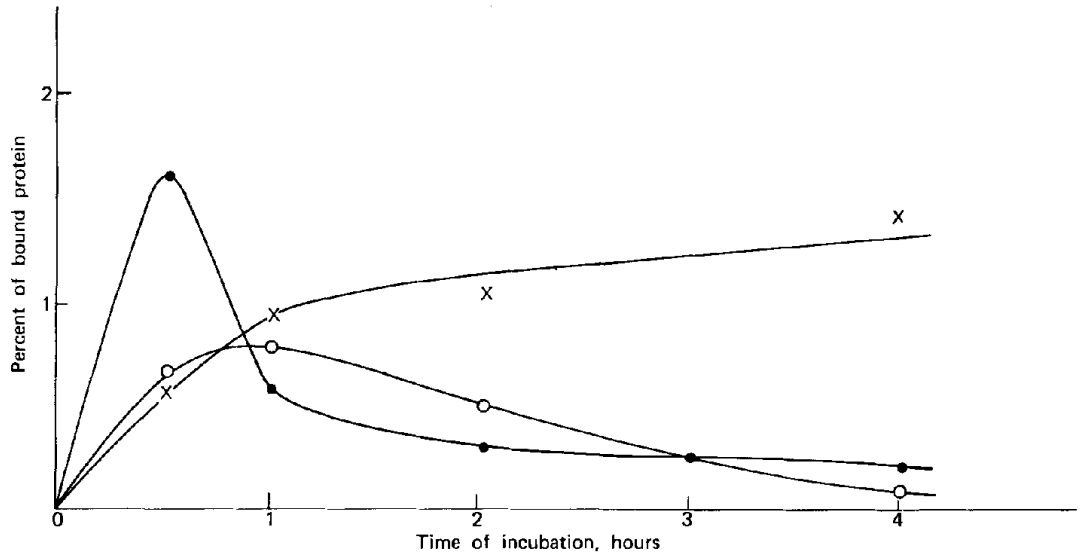


Fig. 1. The effect of bisulfite treatment of MS2 bacteriophage on the amount of labelled protein remaining bound to RNA after phenol deproteinization on TLC plates [12]. 100% is the difference between the radioactivities of the spot before and after deproteinization of the untreated phage. 1 M HSO_3^-, 30°C, (X—X—X) pH 7.0, (•—•—•) pH 6.0, (o—o—o) pH 5.3.

ture was subjected to gel-filtration on Sephadex G-200 (20 X 3 cm column, 0.2 ml/min) in 7 M urea–1% SDS. The absorbancy of the fractions was monitored at 260 nm, and the radioactivity of 0.1 ml aliquots counted in a dioxan scintillator using an SL-30 (Intertechnique, France) scintillation spectrometer; the results are presented in fig. 2.

The RNA-containing fractions were pooled together and precipitated with ethanol, and the precipitate treated with pancreatic RNAase (20 μg RNAase per 5 A_{260} units RNA in 40 μl 3.5 M urea – 5×10^{-3} M Tris–HCl pH 7.6, 60°C, 30 min). The digest was heated for 10 min at 60°C after the addition of 0.8 mg β-mercaptoethanol, SDS to 1%, and urea to 7 M.

The reaction mixture was supplemented with sucrose and bromphenol blue and subjected to electrophoresis on 10% polyacrylamide gel in 1% SDS. Both the cathode and the anode buffers were 1% solution of SDS in 7 M urea. After the electrophoresis (10 V/cm, 5 mA per tube) the gel was dispersed with a Savant device (1 ml 1% SDS per 0.5 ml of gel). In 2 hr of soaking, 10 ml of dioxan scintillator were added per 1 ml of gel suspension and the radioactivity counted at 30% efficiency. The results are presented in fig. 3.

3. Results and discussion

Treatment of MS2 phages with *N*-[^{14}C]acetoxysuccinimide both in the presence and in the absence of bisulfite results in predominant labeling of the phage proteins: of the label incorporated, only about 1% is bound by phage RNA [12]. Hence, it was believed that the *N*-[^{14}C]acetoxysuccinimide method of protein determination [10] would be sensitive enough to detect proteins cross-linked covalently with phage RNA.

To remove the non-bound proteins from RNA, two methods were employed, viz, phenol deproteinization on thin-layer DEAE-cellulose plates [12], and gel-filtration on Sephadex G-200 in 7 M urea–1% SDS. The two methods gave similar results – it was found that the RNA fraction incorporated 1500–2000 cpm per A_{260} unit using the intact phages, and 5000–7000 cpm per A_{260} unit using the phages treated with bisulfite.

To identify the covalently bound proteins, the phage RNA isolated as described above by gel-filtration was digested with RNAase and the digest subjected to gel-electrophoresis. The results of an electrophoretic run are shown in fig. 3. The pattern shown,

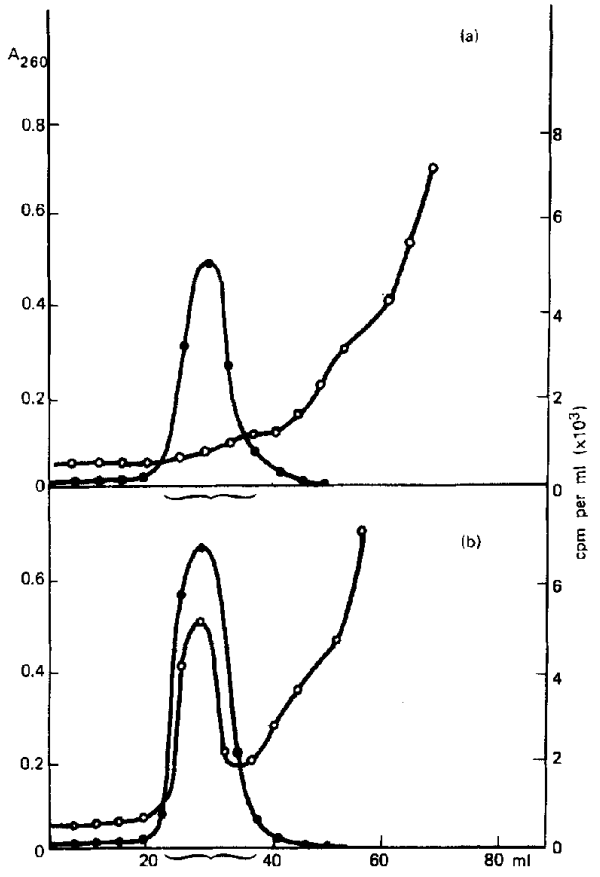


Fig. 2. Gel-filtration on Sephadex G-200 of ^{14}C-labelled MS2 phage preincubated with bisulfite (1 M, pH 7.0, 30°C) for 1–2 min (a) and for 4 hr (b). The conditions are outlined under Materials and methods. (•—•—•) A_{260}, (o—o—o) radioactivity.

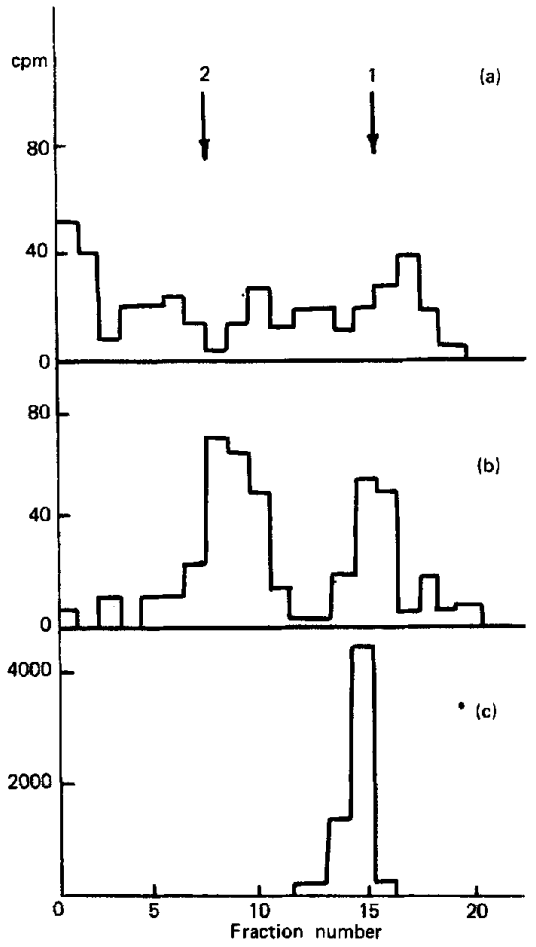
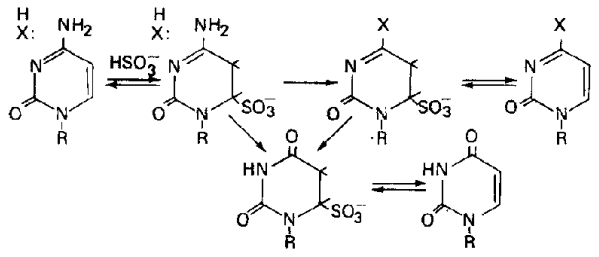


Fig. 3. Gel-electrophoreticpatterns of the proteins of MS2 phage labelled with *N*-[^{14}C]acetoxysuccinimide: (a) fraction marked by a brace in fig. 2a; (b) fraction marked by a brace in fig. 2b; (c) intact phage. In (a) and (b) samples of equal radioactivity were analyzed. The fractions were digested with RNAase as described in Materials and methods. Arrows indicate the positions of coat (1) and maturation (2) proteins visualized by dansylation prior to electrophoresis.

clearly demonstrates that both the coat and the maturation proteins are involved in the crosslinkage with phage RNA.

Earlier we proposed [4] the following scheme of twe bisulfite-induced formation of protein–polynucleotide crosslinks in nucleoproteins:



The following evidence obtained in the course of the present studies is in good accord with this scheme: (i) it was found earlier [4] that at pH 7.2–7.4 bisulfite induced transamination rather than deamination of cytosine residues. As seen in fig. 1, treatment of MS2 phages with bisulfite at pH 7.0 results in incorporation of radioactivity into RNA which remains at a constant level for a long time; (ii) it was found earlier that decrease of pH leads to an increase olf the rate of transamination, but increases also the rate of the deamina-

tion reaction [4, 15, 16]. In line with this, it is seen in fig. 1 that bisulfite treatment of MS2 phages at pH 6.0 results in an increase of the amount of bound protein in the initial period of reaction, while more prolonged treatment leads to a decrease of the incorporation of radioactivity. As for the reaction run at pH 5.3, this results in a small increase of the incorporation level in the initial period, while after 4 hr incubation practically no protein remains bound to RNA (fig. 1).

The data presented in fig. 1 demonstrate that only a limited number [4, 5] of protein molecules is involved in the bisulfite-induced crosslinkage with RNA even under the conditions (1 M bisulfite, pH 7, 20°C) which provide minimum deamination of cytosine nuclei. It may be concluded on this basis that only the cytosine residues which are brought by non-covalent interactions into forced contact with nucleophilic groups of the phage protein amino acid residues are involved in the crosslinkage reaction. Noteworthy in this connection is the fact that the reaction within the phage particle proceeds about a hundred times faster than with model monomer mixtures (fig. 1, cf. ref. [4]).

The transformation of intra-phage non-covalent interactions into covalent bonds provides a means for studying the higher structure of the phage nucleoproteins and for identification of the interacting polynucleotide and protein fragments.

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