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SCHWYZER, Martin, et al.

Abstract

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Reference

Amino Acid Sequence Analysis of Fragments Generated by Partial Proteolysis from Large Simian Virus 40 Tumor Antigen*

Martin Schwyzer,‡§ Roger Welli,‡ Gerhard Frank,‡ and Herbert Zuber‡

From the ‡Department of Molecular Biology, University of Geneva, 1211 Geneva, Switzerland and §Institute of Molecular Biology and Biophysics, Swiss Federal Institute of Technology, 8083 Zurich, Switzerland

Large simian virus 40 tumor antigen was bound as immune complex to protein A-Sepharose and then subjected to limited proteolysis which yielded several discrete fragments. Primary structures near the cleavage sites were determined by radiolaequencing techniques. Experimental data for five fragments matched an amino acid sequence predicted from a nucleotide sequence at 0.51 map unit of the viral genome. We have thus identified the reading frame of translation beyond the intervening sequence at 0.60 to 0.53 map units. A cleavage map of tumor antigen was established on the basis of the sequence data and of the apparent molecular weights of the fragments. The bond most susceptible to cleavage by trypsin was between arginine-130 and lysine-131 in a cluster of five basic amino acids. Other cleavage sites were located in the COOH-terminal half of tumor antigen. Each fragment was analyzed by complete tryptic proteolysis and peptide mapping on an ion exchange column. Peaks occurring in the peptide map of large tumor antigen could thus be assigned to different segments of the protein. Two specific regions of tumor antigen were shown to be phosphorylated.

The early region of the genome of simian virus 40, which is expressed before the onset of viral DNA replication, contains most of the information for lytic infection and apparently all of the information for abortive infection, induction of mitosis, initiation and maintenance of cell transformation, and tumor formation in animals. It comprises about half of the viral genome and directs synthesis of large (M, = 88,000) and small (M, = 45,000) tumor antigens. Study of T-antigen structure and function may be the key to understanding the biological effects of the early region that are thought to be mediated by these proteins (1).

Large and small T-antigens were isolated by immunoaffinity chromatography on staphylococcal protein A-Sepharose. In a further application of this technique, large T-antigen has been cleaved into fragments while bound to the adsorbent. Primary structures near the newly generated NH2 termini have been determined and compared with those deduced from the known nucleotide sequence of SV40 DNA (2, 3). A cleavage map has been established that permits to localize post-translational modifications of the T-antigen protein.

RESULTS

Cleavage of 88,000-dalton T-antigen by Limited Tryptic Proteolysis—Attempts1 to determine amino acid sequences at the NH2 termini of T-antigens isolated from SV40-infected CV-1 cells or synthesized in a reticulocyte lysate primed by mRNA isolated from SV40-infected mouse kidney cells were unsuccessful, suggesting that the NH2 termini were chemically blocked. Paucha et al. (16) have established partial amino acid sequences near the NH2 termini of large and small T-antigens synthesized in vitro under conditions precluding this blockage. In this report, we describe specific and reproducible cleavage of large T-antigen into several fragments enabling us to extend structural analysis to the interior of the T-antigen protein.

Taking advantage of the protein A-Sepharose technique described in the miniprint supplement,2 we subjected T-antigen to limited proteolysis while it was bound to the adsorbent, prior to denaturation with sodium dodecyl sulfate. We expected to obtain by this treatment specific T-antigen fragments of relatively large size, because most potential cleavage sites might be protected from proteolytic attack either by bound antibodies, or by the three-dimensional structure of the T-antigen.

The cleavage patterns shown in Fig. 1 confirmed this expectation. During incubation at 0°C of immune complexes with trypsin, large T-antigen (M, = 88,000) gradually disappeared, and smaller protein species appeared, the most prominent of which (M, = 71,000, 67,000, 58,000, 42,000, 40,000, and 17,000) were derived from large T-antigen, as will be shown below. The species of M, = 45,000 was not related to T-antigen, as shown by fingerprint analysis. Presumably it was actin, which was present in small amounts in most but not all samples. Small T-antigen (M, = 19,000) was degraded more slowly than large T-antigen, and its degradation products have not yet been examined. Gel 1f shows that after 2 h of trypsin treatment both heavy and light chains of immunoglobulin G remained undegraded.

Fig. 2 illustrates the procedure by which T-antigen fragments were isolated. T-antigen from SV40-infected CV-1 cells

1 Portions of this paper (including "Materials and Methods" and part of "Results") are presented in miniprint at the end of this paper. The abbreviation used is: PTH, phenylthiohydantoin. Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Maryland 20014. Request Document 79M-1915, cite authors, and include a check or money order for $1.80 per set of photocopies.

2 M. Schwyzer, J. Gagnon, L. H. Ericsson, and K. A. Walsh, unpublished observations.

3 M. Schwyzer, unpublished observations.

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‡ To whom reprint requests should be sent.

§ The abbreviations used are: T-antigen, tumor antigen. SV40, simian virus 40.
obtain an electrophoretically homogeneous preparation of the 40,000-dalton fragment, which was free of detectable 42,000-dalton material. Fragments were mixed with β-lactoglobulin and subjected to automated NH₂-terminal sequence analysis. Radioactivity associated with the thiazolinones obtained in each degradative cycle was determined by liquid scintillation counting. Since only two amino acids containing two different isotopes were labeled, they could be identified without prior chromatographic separation (see below).

As shown in Fig. 3, peaks of ³H radioactivity were released from the 40,000-dalton fragment at cycles 8, 15, 21, and 29. About 36% and 14% of the peak radioactivity was released in the cycles preceding and following each peak cycle, respectively. This may indicate microheterogeneity at the NH₂-terminus of the 40,000-dalton fragment (see below). From one peak to the next, radioactivity decreased regularly; repetitive yields calculated for the three intervals between peaks were 93, 94, and 94%, respectively, indicating that degradation had proceeded smoothly. Radioactivity at cycle zero was calculated to be 2500 cpm/residue, which was about 60% of the value expected from the known input radioactivity. No peaks

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**Fig. 1.** Time course of limited tryptic proteolysis of T-antigen. Aliquots of [³S]methionine-labeled T-antigen bound to protein A-Sepharose were subjected to limited tryptic proteolysis and then analyzed by gel electrophoresis as indicated under "Materials and Methods." except that the proteolysis time was varied: a, 0 min; b, 15 min; c, 30 min; d, 60 min; e and f, 120 min. a to e, autoradiography; f, Coomassie blue staining showing the heavy (H) and light (L) chains of immunoglobulin G.

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**Fig. 2.** Isolation of T-antigen fragments obtained by limited tryptic proteolysis. At different stages of the procedure (see "Materials and Methods"), aliquots were analyzed by electrophoresis on 7.5% polyacrylamide gel. a, T-antigen before proteolysis; b, material released into wash buffer after tryptic proteolysis; c, fragments retained by the column after tryptic proteolysis and released by sodium dodecyl sulfate; f to i, re-electrophoresis of T-antigen fragments isolated from preparative gels; d, material released into wash buffer; e, fragments of T-antigen prepared from SV40-infected mouse kidney cell cultures; all others from SV40-infected CV-1 cells.

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**Fig. 3.** Edman degradation of 40,000-dalton fragment of SV40 T-antigen labeled with [³H]phenylalanine and [³S]methionine. ³H radioactivity released at each cycle was determined according to "Materials and Methods." ³S radioactivity was 30 cpm or less per cycle and is not shown here. The sequence at the top is explained in Fig. 4.
of $^{35}$S radioactivity were observed although the fragment was sufficiently labeled with $[^{35}S]$methionine (500 cpm/residue) to have permitted detection of a peak with at least twice the background radioactivity in cycle 32.

More extensive sequence data were obtained by analyzing a mixture of the 40,000- and 42,000-dalton fragments, both labeled with tritiated proline, leucine, valine, phenylalanine, lysine, and with $[^{38}S]$methionine; the 40,000-dalton fragment contained about 70% of the total radioactivity. Thiazolinones released at each cycle were converted to phenylthiohydantoin-amino acids and separated by high pressure liquid chromatography. Labeled material eluted with each phenylthiohydantoin-amino acid was quantitated by liquid scintillation counting.

As shown in Fig. 4, peaks of proline were released at cycles 5 and 9, of phenylalanine at cycles 8, 15, 21, and 29 (Fig. 4, upper), of leucine at cycles 12, 13, 16, and 26, of lysine at cycles 1 and 6 (Fig. 4, middle), and of valine at cycle 2 and possibly cycle 20 (Fig. 4, lower). No peak of $^{35}$S radioactivity was detected. At the top of Fig. 4 is shown an amino acid sequence (one letter code) predicted from a nucleotide sequence (2, 3) located at 0.51 map units of the SV40 genome. The 14 amino acid residues that we have identified (solid arrows) exactly match the predicted sequence. Under the conditions of limited tryptic proteolysis described here, cleavage must have occurred between arginine-130 and lysine-131 (broken arrow) in a cluster of five basic amino acid residues. The data also suggested that the 42,000-dalton fragment present in the mixture had the same NH$_2$ terminus as the 40,000-dalton fragment.

Radioactivity of each phenylthiohydantoin-amino acid was significantly higher in the cycles preceding and following each peak, as compared to other cycles. This was probably not due to asynchronous degradation, since very little asynchrony was detected in the concomitant degradation of $\beta$-lactoglobulin. The data are consistent with the assumption that about 65% of T-antigen molecules were cleaved between arginine-130 and lysine-131 and 25% and 10% at adjacent peptide bonds to the left and the right, respectively.

As discussed in the miniprint supplement, the 58,000-, 67,000-, and 71,000-dalton fragments were found to have the same NH$_2$ terminus as the 40,000-dalton fragment.

**Fingerprint Analysis of Fragments of Large T-antigen—**

Lichaa and Niesor (7) analyzed tryptic peptides of $[^{35}S]$methionine-labeled large SV40 T-antigen by ion exchange chromatography. A more detailed analysis, including chromatography of $[^{3}H]$leucine-labeled peptides, will be published elsewhere. Here we demonstrate that the rather complex peptide map of 88,000-dalton T-antigen can be subdivided into simpler maps by the separate analysis of T-antigen fragments.

Fig. 5 shows, as an example, the chromatographic analysis of $[^{35}S]$methionine-labeled (a and c) and $[^{3}H]$leucine-labeled (b and d) tryptic peptides of the 71,000-dalton fragment (a and b) and the 17,000-dalton fragment (c and d) of large T-antigen. Each of the T-antigen fragments was analyzed several times in this way. The results are summarized in Table I in which lists the $^{35}$S and $^3$H radioactivity contained in each peak of the peptide maps of large T-antigen and its fragments.

We observed that the peptide maps of the 71,000- and 17,000-dalton fragments were not related to each other. However, the sum of the two maps closely matched the peptide map of large T-antigen (Table I). This was consistent with the assumption that the two fragments were derived from large T-antigen by a single cleavage at the site illustrated in

Fig. 4. Edman degradation of a mixture of 40,000- and 42,000-dalton fragments of SV40 T-antigen labeled with $[^{14}N]$$\beta$phenylalanine, $[^{14}N]$proline, $[^{14}N]$leucine, $[^{14}N]$lysine, $[^{14}N]$valine, and $[^{35}S]$methionine. $^3$H radioactivity released at each cycle and associated with the phenylthiohydantoins of these amino acids was determined according to "Materials and Methods." As control, $^3$H radioactivity associated with the phenylthiohydantoins of alanine, glycine, and tryptophan was determined and found to be less than 10 cpm/cycle. $^{35}$S radioactivity gradually increased during degradation and reached 50 cpm at cycle 30. No peaks of $^3$H radioactivity were detected at cycles 18, 22 to 25, 28, 31, and 32, determined without chromatographic separation. At the top is the predicted amino acid sequence of large SV40 T-antigen (resides 127 to 162, see text).

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COOH-terminal region of the 17,000-dalton fragment and the NH2-terminal region of the 58,000-dalton fragment overlapped, because the 40,000-dalton fragment lacked peptides 3.73 and 3.99 although having the same NH2 terminus. Furthermore, since peptides "a" and "b" differed strongly in composition, they were probably unrelated to each other and co-migrated by chance.

Surprisingly, the 58,000- and 71,000-dalton fragments gave rise to identical peptide maps. Thus, a substantial part of the COOH-terminal region of large T-antigen (0.24 to 0.17 map unit; about 120 amino acid residues) did not contribute any recognizable peaks to the peptide maps, although this region should contain 3 methionine and 4 leucine residues according to the predicted sequence. It remains unknown whether these peptides were absent because of their chemical properties (insolubility, acidity), or because the COOH-terminal region was degraded incompletely by trypsin.

Cleavage Map of Large SV40 T-antigen—On the basis of the data presented here, a cleavage map of the large T-antigen protein (Fig. 6) was established. The top of the scheme shows the early region of the SV40 genome (0.67 to 0.15 map unit) and one of its transcripts, from which the region of 0.60 to 0.53 map unit has been removed by splicing (17, 18). The spliced mRNA species codes for the large T-antigen protein,
TABLE I

Fingerprint analysis of several fragments of large T-antigen labeled with [35S]methionine and [3H]leucine

Fragments were analyzed as described in the legend of Fig. 5. Analysis of 88,000-dalton T-antigen is given for comparison. Results are averages of several analyses and are listed as relative amount of radioactivity (in %) contributed by each peak (sum of peaks derived from large T-antigen = 100%). Peaks labeled with superscripts a or b co-migrate in the map of large T-antigen but are derived from different fragments.

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Fig. 6. Early region of SV40 DNA, spliced early mRNA coding large T-antigen, and cleavage map of large T-antigen. *NH, terminus of large T-antigen (presumably blocked). Positions of COOH termini are based on apparent molecular weights and must be regarded as approximative. P-T, possible phosphorylation sites (see Fig. 7 and text). For other details see text and miniprint supplement.

which is drawn here as a line along the coding region of the RNA. T-antigen fragments are drawn as similar lines along those regions of large T-antigen from which, according to our data, they originated.

To illustrate how the present cleavage map might aid further analysis of T-antigen, the experiment shown in Fig. 7 was performed, where T-antigens or fragments thereof, labeled with [35S]methionine or [32P]orthophosphate (13), were compared side by side. Gel a shows electrophoretic analysis of a standard [35S]methionine-labeled T-antigen preparation; large T-antigen, two of its presumed breakdown products (M, = 83,000 and 78,000), actin, and small T-antigen were recognized by autoradiography. In contrast, in the preparation labeled with [32P]phosphate, only the large T-antigen and the 83,000- and 78,000-dalton species were observed. Small T-antigen did not appear to be labeled with [32P], although the presence of a very diffuse band of [32P] radioactivity in the 17,000 to 25,000-dalton region (not noticeable in this photograph) might have obscured a weakly labeled small T-antigen. To investigate how [32P] radioactivity was distributed over the different fragments, [32P]-labeled T-antigen was subjected to limited trypptic proteolysis (gel d). Comparison with standard [35S]-labeled fragments (gel c) revealed that the bulk of [32P] radioactivity migrated with the 17,000-dalton fragment, whereas the 71,000-, 67,000-, and 58,000-dalton fragments contained comparatively small amounts of [32P] radioactivity. The 40,000- and 42,000-dalton fragments did not contain any detectable [32P] radioactivity.

T-antigen was also subjected to limited proteolysis by staphylococcal protease under conditions where the enzyme cleaves after glutamic and aspartic acid residues. When this type of proteolysis was performed at 0°C (gel e), large and small T-antigen remained essentially undegraded, except for the appearance of two fragments (M, = 75,000 and 60,000) in small amounts. Proteolysis at 25°C gave rise to the patterns shown in gels f and g. The bulk of [32P] radioactivity (gel g) migrated with the 60,000-dalton fragment, whereas the 71,000-, 67,000-, and 58,000-dalton fragments contained comparatively small amounts of [32P] radioactivity. The 40,000- and 42,000-dalton fragments did not contain any detectable [32P] radioactivity.

The results shown in Fig. 7 were interpreted according to the cleavage map of large T-antigen. Since the bulk of [32P] radioactivity was associated with the 17,000-dalton fragment, we concluded that segment P (0.65 to 0.60 map unit) or segment Q (0.53 to 0.51 map unit) was phosphorylated, and constitutes the NH2-terminal region of both large and small T-antigen, whereas segment Q is present in large T-antigen only. The fact that no detectable [32P] radioactivity was associated with small T-antigen suggested that segment P did not contain any phosphorylation sites and that segment Q was the principal phosphorylated region of large T-antigen. Alternatively, segment P was phosphorylated in large T-antigen but not in small T-antigen because of differences in confor-
mation or subcellular location. It should be noted that serine and threonine residues, which are potential phosphorylation sites, are more abundant in segment Q (9 out of 48) than in segment P (4 out of 52).

The presence in large T-antigen of an additional phosphorylated region comprising segments R, S, and T (0.32 to 0.17 map unit; Fig. 6) was deduced from the fact that the 58,000-, 67,000-, and 71,000-dalton fragments were phosphorylated, whereas the 40,000-dalton fragment was not. The data did not allow to establish whether phosphorylation was restricted to segment R or occurred also in segments S and T. The chemical nature of the different $^{32}$P-labeled residues remains to be determined.

Discussion

Establishment of a cleavage map of SV40 DNA (19) marked a crucial step in virus research, culminating 5 years later in the elucidation of the complete structure of the SV40 genome (2, 3). In analogy, if a protein as large as T-antigen ($M_0 = 88,000$) is to be analyzed in any structural detail, it must first be subdivided into fragments that can be analyzed separately. T-antigen was isolated in the present work by immunofluorescence and protein A-Sepharose (12) in good yield (approximately 2 $\mu$g of T-antigen protein/culture of 15 $\times$ 10$^7$ CV-1 cells) and high radiochemical purity. Our method gives similar results to those obtained by immunoprecipitation with protein A-bearing bacteria (15, 20), and has been used successfully for isolation of other proteins (21-23).

To generate fragments of large T-antigen, immune complexes were bound to protein A-Sepharose and then subjected to limited proteolysis. According to our results, trypsin cleaves large T-antigen preferentially at one specific peptide bond, between arginine-130 and lysine-131 (0.51 map unit), located in the NH$_2$-terminal half of the protein in a cluster of five basic amino acid residues. Additional cleavage sites, which are less susceptible to trypsin, are located exclusively in the COOH-terminal half of T-antigen. This suggests that a large part of T-antigen is protected from proteolytic attack by bound antibodies or by its own three-dimensional structure. Therefore, the cleavage technique described here appears to be more selective than a technique subjecting denatured proteins to proteolysis (24). The amino acid sequence data reported here were obtained by automated Edman degradation of T-antigen fragments (about 20 pmol/analysis) that had been labeled by the addition of five different radioactive amino acids to the culture medium during infection. These amino acid residues were unambiguously identified for up to 29 Edman degradation cycles by high pressure liquid chromatography and scintillation counting (26). No additional radioactive phenylthiohydantoin-amino acids were detected, indicating that under the conditions used the metabolic interconversion of labeled amino acids was negligible. The positions of the 14 amino acid residues that we have placed in sequence agree with an amino acid sequence predicted from a DNA nucleotide sequence at 0.51 map unit of the viral genome (2, 3). We have thus identified the reading frame of translation beyond the noncoding sequence at 0.60-0.53 map unit (17). Considering the results obtained in other laboratories (16-18, 25), the primary structure of large SV40 T-antigen appears to be established. Nevertheless, the possibility remains that additional as yet undetected splices or errors in the determined nucleotide sequence would cause translation outside the predicted reading frame for part of the length of T-antigen. It should be noted in this context that the amino acid sequences predicted by Reddy et al. (18) and Fiers et al. (3) differ in positions 522, 531, and 549.

Tegtmeyer et al. (13) showed that a single tryptic peptide of large T-antigen contained phosphoserine, and that the presence of additional, acid-labile phosphate groups could not be excluded. The present report demonstrates that at least two specific regions of T-antigen are phosphorylated. According to the cleavage map shown in Fig. 6, one phosphorylated region comprises segment Q and possibly segment P near the NH$_2$ terminus, and the other comprises segment R and possibly segments S and T near the COOH terminus of large T-antigen. This finding serves to illustrate that certain aspects of T-antigen structure cannot be deduced from the viral nucleotide sequence alone but must be elucidated by direct chemical analysis. The dissection of T-antigen described here will facilitate this analysis and should contribute to the understanding of its function.

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Conversion of SV40 T-antigen into viral DNA. The thymidines released at each replication cycle were mixed with unlabeled thymidines released at the previous cycle and treated with 2 M NaCl to form ethyl acetate and dried in a stream of nitrogen, as described (12). T-labeled T-antigens were identified by high-performance liquid chromatography (HPLC) with the following modifications to permit analysis of radioactively labeled amino acid derivatives. The dried T-labeled T-antigens were dissolved in 300 μl of the reaction buffer (50 mM Tris-Cl, pH 7.5, 0.5 M NaCl, 0.5 M MgCl2, 20 mM dithiothreitol, and 0.5% NP40). The reaction was initiated by adding 30 μl of the T-labeled T-antigen solution and 10 μl of a 1:10 dilution of the enzyme solution, and the mixture was incubated for 3 h at 37°C. The reaction was stopped by the addition of 10 μl of 10% trichloroacetic acid and 100 μl of 1% sodium dodecyl sulfate (SDS). The samples were analyzed by high-performance liquid chromatography (HPLC) on a Shiseido (Tokyo) LC-6A with an ultraviolet detector at 254 nm. The elution of T-antigen fractions was monitored by the absorbance at 254 nm, and the fractions containing the T-antigen were collected and used for the following experiments.

RESULTS

Isolation of T-antigens by Immunofluorescence Chromatography

T-antigens were isolated by immunofluorescence chromatography (Figure 1). T-antigens were eluted from the immunoaffinity column with a sample buffer containing 100 mM Tris-Cl, pH 7.4, followed by immunoprecipitation with a lower apparent molecular weight than T-antigens isolated from S. aureus. T-antigens were eluted from the immunoaffinity column with a sample buffer containing 100 mM Tris-Cl, pH 7.4, followed by immunoprecipitation with a lower apparent molecular weight than T-antigens isolated from S. aureus. T-antigens were eluted from the immunoaffinity column with a sample buffer containing 100 mM Tris-Cl, pH 7.4, followed by immunoprecipitation with a lower apparent molecular weight than T-antigens isolated from S. aureus. T-antigens were eluted from the immunoaffinity column with a sample buffer containing 100 mM Tris-Cl, pH 7.4, followed by immunoprecipitation with a lower apparent molecular weight than T-antigens isolated from S. aureus.

Radioactivity in each fraction was determined and found to represent 20-40% of the total radioactivity associated with each fraction. This result was consistent with the observation that the T-antigen band in the gel had a higher molecular weight than the T-antigen band in the gel.

Fig. 1. Effect of the sample buffer on extraction of T-antigen fragments. T-antigens were isolated from S. aureus-infected monkey or mouse kidney cell cultures. The T-antigen band in the gel had a higher molecular weight than the T-antigen band in the gel.

Isolation of T-antigens by Immunofluorescence Chromatography

T-antigens were isolated by immunofluorescence chromatography (Figure 1). T-antigens were eluted from the immunoaffinity column with a sample buffer containing 100 mM Tris-Cl, pH 7.4, followed by immunoprecipitation with a lower apparent molecular weight than T-antigens isolated from S. aureus. T-antigens were eluted from the immunoaffinity column with a sample buffer containing 100 mM Tris-Cl, pH 7.4, followed by immunoprecipitation with a lower apparent molecular weight than T-antigens isolated from S. aureus. T-antigens were eluted from the immunoaffinity column with a sample buffer containing 100 mM Tris-Cl, pH 7.4, followed by immunoprecipitation with a lower apparent molecular weight than T-antigens isolated from S. aureus. T-antigens were eluted from the immunoaffinity column with a sample buffer containing 100 mM Tris-Cl, pH 7.4, followed by immunoprecipitation with a lower apparent molecular weight than T-antigens isolated from S. aureus.
**Cleavage Map of SV40 T-antigen**

**Sequence analysis of SV40 T-antigen fragments**

Fig. 10 shows sequence analysis of the 58,000 and 71,000-dalton fragments obtained from T-antigen labeled with [35S]methionine. The 58,000-dalton fragment, labeled with [35S]methionine, was subjected to two cycles of digestion with trypsin, and the resulting peptides were analyzed by immunoaffinity chromatography. The results obtained from these experiments are shown in Fig. 10. The 58,000-dalton fragment was found to contain a single tryptic peptide, designated T2, which was found to be identical to the sequence of T-antigen located at position 125 to 145.

Fig. 11 shows sequence analysis of the 69,000-dalton fragment of T-antigen labeled with [35S]methionine. The 69,000-dalton fragment was subjected to three cycles of digestion with trypsin, and the resulting peptides were analyzed by immunoaffinity chromatography. The results obtained from these experiments are shown in Fig. 11. The 69,000-dalton fragment was found to contain three tryptic peptides, designated T3, T4, and T5, which were found to be identical to the sequences of T-antigen located at positions 146 to 160, 161 to 175, and 176 to 190, respectively.

**Fig. 7** Determination of optical conditions for immunoaffinity chromatography and gel electrophoresis. The immunoaffinity chromatography and gel electrophoresis were conducted according to the method of M. S. W. H. and T. L. M. methionine. The resulting eluates were analyzed by SDS-PAGE, and the results are shown in Fig. 7. The immunoaffinity chromatography was found to be effective in separating the T-antigen from other proteins, and the gel electrophoresis was found to be effective in separating the T-antigen from other polypeptides.

**Fig. 12** Determination of analytical conditions for immunoaffinity chromatography and gel electrophoresis. The immunoaffinity chromatography and gel electrophoresis were conducted according to the method of M. S. W. H. and T. L. M. methionine. The resulting eluates were analyzed by SDS-PAGE, and the results are shown in Fig. 12. The immunoaffinity chromatography was found to be effective in separating the T-antigen from other proteins, and the gel electrophoresis was found to be effective in separating the T-antigen from other polypeptides.