Interspecies Homology of RNA Tumor Virus Proteins†

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ABSTRACT: We report the application of a highly sensitive column chromatographic technique to the comparison of tryptic peptide maps of some RNA tumor virus proteins. By combining microbore ion-exchange chromatography with a sensitive fluorescent assay using *o*-phthalaldehyde, we obtained high-resolution peptide maps starting with only microgram amounts of protein. Our discovery of coincident peptides from the 15 000 and 30 000 molecular weight proteins from murine and feline leukemia viruses supports serological evidence for interspecies antigenic determinants; coincident peptides were also found for the 10 000 molecular weight proteins from these viruses, although immunochemical data did not reveal interspecies determinants. The relatively large number of coeluting peptides found in the 15 000 and 10 000 molecular weight proteins is strong evidence for the existence of homology.

Among the most interesting proteins from C-type RNA viruses are those containing antigens common to other oncogenic viruses. Two types exist: "group-specific antigens" common to different viruses hosted by a given animal species and "interspecies antigens" belonging to C-type viruses of differing animal species. Group specific antigens have been studied by several investigatators (Fleissner, 1971; Hung et al., 1971; Nowinski et al., 1972; Schäfer et al., 1971, 1972), but most recent attention has focused on interspecies antigens. The major internal polypeptide of C-type viruses (27 000 to 30 000 molecular weight) contains such an interspecies antigen, first reported by Geering et al. (1970). It has been purified and extensively described (Schäfer et al., 1971, 1972; Gilden et al., 1971; Oroszlan et al., 1972, 1971; Parks and Scolnick, 1972; Sherr et al., 1975; Strand and August, 1975). At least three other components of mammalian C-type viruses also contain interspecies antigens. Antibody to RNA-dependent DNA polymerase from either murine or feline virus inhibits polymerase activity of virus hosted by hamster, rat, mouse, and cat (Aaronson et al., 1971; Scolnick et al., 1972); it thus appears that this viral polymerase contains an interspecies determinant. The 69 000 and 71 000 molecular weight glycopeptides of mammalian tumor viruses contain interspecies antigens (Strand and August, 1973, 1974a) and the 15 000 molecular weight polypeptide from murine leukemia virus (MuLV)[1] was also recently shown to have interspecies reactivity (Schäfer et al., 1975; Strand et al., 1974).

Existence of common antigenic determinants suggests the existence of regions of sequence identity within the protein molecules. Oroszlan and co-workers reported the sequence of the first several amino acids in the major internal protein from feline, murine, and other C-type viruses and found significant areas of identity within the portions examined (Oroszlan et al., 1973, 1974). Existence of large regions of sequence identity suggests a common functional role of those proteins and implies existence of a common viral ancestry. Either some prototype virus infected and established itself in one species and spread to others, or the virus and host coevolved from some distant period. The "oncogene theory" of coevolution (Huebner and Todaro, 1969) suggests that the viral genome has become integrated into host DNA, exists in every cell, and is vertically transmitted.

This theory is supported by evidence that oncogenic viral antigens are present in cells containing neither infectious virus nor virus particles (Gilden and Oroszlan, 1972). Recently, cell extracts from cancer patients were found to contain one or more proteins of approximately 30 000 molecular weight that bound to antibodies directed against core proteins from wooly monkey and RD114 viruses (Strand and August, 1974b). This implies that C-type viral proteins exist in human tissue. Thus, suggestions as to the viral etiology of human cancer coupled with implications regarding taxonomy and evolution of tumor viruses make studies of molecular structure of viral proteins containing common antigenic determinants particularly important.

Amino acid sequence determination requires substantial quantities of purified protein but, typically, only microgram amounts of oncogenic viral proteins are available. Since proof of homology requires determination of primary structure, additional quantities of material or more highly sensitive methods of sequencing are necessary. It is possible, however, to obtain presumptive evidence of protein homology without sequence determination; for example, through tryptic peptide mapping.

We report here the application of a new, highly sensitive chromatographic technique to the comparison of tryptic peptide maps of some RNA tumor virus proteins obtained from viruses of different animal species. Starting with only microgram amounts of purified material, we found evidence of regions of relatedness among proteins reported to contain interspecies antigenic determinants and also in the 10 000 molecular weight proteins from feline and murine leukemia viruses, although these are reported to contain no common antigens (Green et al., 1973; Schäfer et al., 1975).

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[1] Abbreviations and nomenclature used in this report are: MuLV, murine leukemia virus (Friend); FeLV, feline leukemia virus (Rickard); AMV, avian myeloblastosis virus; OPA, *o*-phthalaldehyde. These abbreviations, followed by the letter "p" and a number, represent the molecular weight, in thousands, of the associated protein. Thus, MuLV p10 represents the 10 000 molecular weight protein from murine leukemia virus.

The technique, first described by Benson (1976), combines microbore ion-exchange chromatography with a new, highly sensitive fluorescent assay for biogenic amines. The fluorescent assay employs *o*-phthalaldehyde (OPA) which in the presence of 2-mercaptoethanol reacts with primary amines to form highly fluorescent products. A similar reagent solution used by Roth (1971) for detection of amino acids was modified by Benson and Hare (1975) for detection of picomole quantities of peptides. We have now utilized the latter reagent in a constant-pressure microbore liquid chromatograph that allowed high-resolution peptide mapping of microgram amounts of enzymatically digested proteins.

Materials and Methods

Virus Proteins. Purified virus proteins (Bolognesi and Bauer, 1970) were generously provided by Dr. Dani Bolognesi at Duke University through the Virus Cancer Program of the National Cancer Institute. In his laboratory, Avian myeloblastosis virus (AMV) was obtained from the blood of leukemic chickens; Friend murine leukemia virus (MuLV) and Rickard feline leukemic virus (FeLV) were grown in tissue culture. The virus proteins he supplied had been purified by agarose gel filtration in guanidine hydrochloride. Those requiring further purification were treated with ammonium sulfate and carboxymethylcellulose ion-exchange chromatography. Purity of individual polypeptides was ascertained by urea gel or sodium dodecyl sulfate gel electrophoresis, by end-group analysis, and by polyacrylamide gel isoelectric focusing. Details of protein purification are given elsewhere (Schäfer et al., 1975; Green and Bolognesi, 1974; Green et al., 1973). Protein content was determined in the laboratory of Dr. Bolognesi by the method of Lowry et al. (1951). The purified 15 000 molecular weight polypeptide from MuLV supplied by Dr. Bolognesi originated from the viral envelope (Ihle et al., 1975). This has been termed p15(E) by Ikeda et al. (1975) to distinguish it from the p15 protein found in the viral core.

The Microbore Chromatograph. The microbore ion-exchange liquid chromatograph utilized constant-pressure eluent pumps modeled after those originally described by Hare (1969, 1972) and is described in detail elsewhere (Benson, 1976). A 57 °C jacketed column containing a 0.20 × 25 cm bed of Durrum DC-4A cation-exchange resin was used (Durrum Chemical Corp., Sunnyvale, Calif.); eluent flow rate was 6.3 mL/h (linear flow velocity = 3.3 cm/min). OPA reagent solution flow rate was also maintained at 6.3 mL/h. Fluorescence was monitored with an Aminco filter fluorometer (American Instrument Co., Silver Spring, Md.) using a Corning 7-60 filter for excitation and a Wratten 2A filter for emission. A Honeywell Electronik 19 strip chart recorder with a chart speed of 3 in./h recorded the fluorometer output using a range setting of 10 mV, full-scale deflection. A Durrum eight-channel programmer controlled the time interval and sequence of buffer solutions and shut off recorder and reagent flow at the end of the analysis.

Water. The water used in all prepared solutions was purified by a commercial deionization system (Aqua Media Co., Sunnyvale, Calif.) in which tap water was prefiltered, passed through an organic sorption cartridge, two mixed-bed ion-exchange cartridges, and into an ultraviolet flow cell before final passage through an 0.2-μm filter. Resistance was continuously monitored with an ohmmeter. Only water with a specific resistance of at least 16 Mohm/cm was used.

Reagent Solutions. OPA reagent solution was prepared from "Fluoropa", a fluorogenic grade of *o*-phthalaldehyde (Durrum Chemical Corp.). The crystals were combined with 2-mercaptoethanol in a pH 10.5 borate buffer solution according to instructions provided with the material.

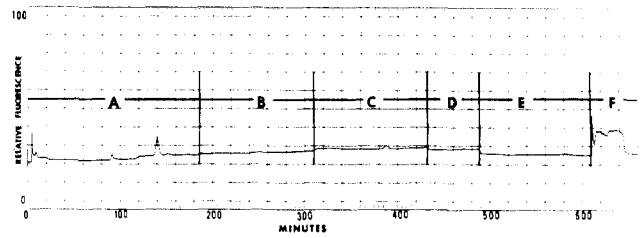

FIGURE 1: Control. Twenty microliters of deionized water was treated with trypsin and analyzed in the same manner as protein samples. Small peaks appearing during the first 20 min are probably amino acids; the peak at approximately 140 min is ammonia. Other small peaks are of unknown origin. The plateau occurring after 600 min results from contaminants deposited on the resin bed by early-eluting buffer solutions that are subsequently eluted by the high-pH solution. The length of time each buffer solution is in the column and the breakthrough of each successive solution are indicated.

Six buffer solutions were used in sequence to provide separation of the tryptic peptides. The first four solutions were prepared from sodium citrate dihydrate (Baker), 19.6 g dissolved in 980 mL of water. One milliliter of liquid phenol (Mallinckrodt AR) was added as a preservative. Reagent grade HCl (Baker) was used to adjust pH values of the four solutions to 4.60, 5.00, 5.35, and 6.25, respectively. The fifth solution was prepared by adding 16 g of 50% NaOH (Baker) plus 1.0 mL of phenol to 980 mL of water. *o*-Phosphoric acid (Baker) was used to adjust pH to 7.20 (ca. 13 g required). The sixth solution was prepared by adding 1.0 mL of phenol, 4.0 g of 50% NaOH, 8.76 g of NaCl and 3.0 g of boric acid (Baker) to 990 mL of water. The solution was adjusted to pH 9.50 with HCl. All solutions were 0.20 M in sodium.

All buffer solutions were filtered through a 2.5-cm diameter Millipore "Polyvic" 0.5-μm pore filter disk. Substances present in these disks are leached by the buffer solutions and react with OPA; to extract these substances, about 2 L of water was passed through each disk before filtering any solutions.

Sample Preparation. Preparation of purified proteins and the tryptic hydrolysis conditions are given in detail elsewhere (Benson, 1976). Typically, 100 μg of purified denatured protein diluted to 500 μL with water maintained at 37 °C was treated with a total of 4 μg of Tos-PheCH_2Cl trypsin (Worthington Biochemicals) at pH 8 to 9. All material was dissolved. The residue after lyophilization of the tryptic peptides was dissolved in 100 μL of 10^{-3} M HCl and stored at −20 °C. All material remained in solution after thawing; a 20-μL aliquot was applied to the resin bed for each analysis. At least two analyses were performed with each sample of tryptic peptides; resultant elution profiles were nearly identical. Two separate tryptic digestions were carried out with the mammalian p30 and p15 virus proteins. No significant differences existed in the resultant elution profiles.

Results

Control. A control vial containing 500 μL of water was treated with trypsin in exactly the same fashion as vials containing protein samples to determine if the sample handling procedure (Benson, 1976) prevented contamination of OPA-reactive substances. Analysis of this control provided a background against which all peptide maps could be compared (Figure 1). The length of time each buffer solution remained in the column is indicated; this program was used for all subsequent analyses. Only a few fluorescent peaks appear during the first 10 min; these are probably amino acids that contam-

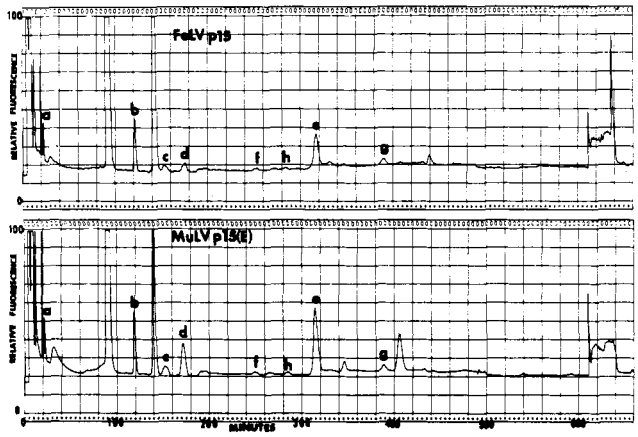

FIGURE 2: Comparison of FeLV p15 and MuLV p15(E). Tryptic peptides from 24 μg each of the p15 proteins from feline and murine leukemia viruses were chromatographed. Coeluting peptides are labeled; peaks eluting at approximately 90 min (resulting from deoxycholate present in the samples) are not considered coincident. Inconsistancies in heights of peaks d, e, and the peak eluting immediately before a are discussed in the text.

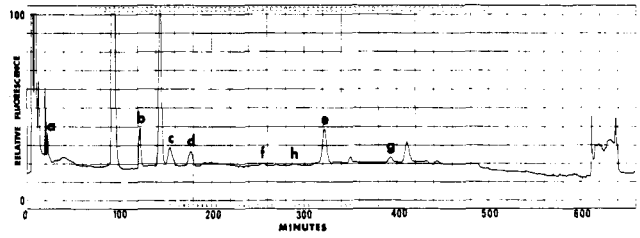

FIGURE 3: Mixture of tryptic peptides from MuLV p15(E) and FeLV p15. To verify that labeled peptides in Figure 2 were indeed coincident and did not coelute as a result of eluent flow-rate variations, a mixture containing tryptic peptides from 12 μg each of the two proteins was chromatographed. Comparison of this profile with those in Figure 2 confirms the relative elution positions and coincidence of the peaks. The fact that peak fluorescent values are slightly less than expected may be the result of a sampling error.

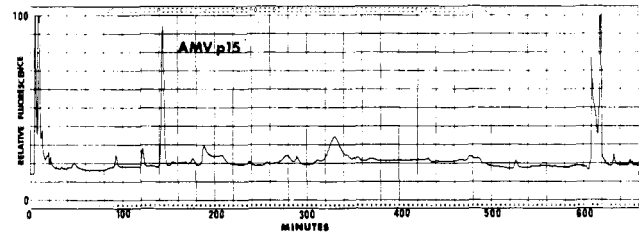

FIGURE 4: AMV p15 tryptic peptides. Tryptic peptides from 24 μg of the p15 protein from avian myeloblastosis virus were chromatographed. Although one peak at approximately 120 min appears to coelute, the resultant pattern is generally dissimilar to those shown in Figure 2. This is consistant with serological experiments that demonstrate no evidence of common antigenic determinants among avian and mammalian species.

inated reagents used in preparing diluents for trypsin solutions or that were picked up from the atmosphere. In an analysis of a calibration mixture of amino acids (data not shown), ammonia appears at approximately 140 min; thus, the small peak at approximately 140 min in Figure 1 is assigned as ammonia. Other significant fluorescent peaks are absent from the chromatogram, suggesting that the sample handling technique sufficiently prevents contamination by OPA-reactive substances.[2] Because the trypsin was present in such small amounts (usually 4% of the total protein), peptides from its self-digestion do not appear at this sensitivity.

Comparison of FeLV p15 and MuLV p15(E). Figure 2 illustrates comparative analyses of tryptic peptides from 24 μg of FeLV p15 and 24 μg of MuLV p15(E). The letters in the two chromatograms denote peaks that coelute. The large, off-scale peak at approximately 90 min results when deoxycholate is present in the sample (Schäfer et al., 1975); the large peak at approximately 140 min is ammonia. Thus, these two peaks are not considered coeluting peptides. Note that peaks labeled d and e and another coincident peak immediately to the left of a in the FeLV p15 map show fluorescent values inconsistant with their counterparts in the MuLV p15(E) map. The fluorescence ratio of b to d from FeLV p15, for example, obviously differs from the corresponding peak ratio from MuLV p15(E).

If a change in eluent flow rate had occurred in the second analysis, elution times of individual peaks would shift and some could wrongly appear to coincide with those in the first analysis. Therefore, to verify that the labeled peaks were indeed coincident, 30 μL (35 μg) of each of the tryptic peptides from MuLV p15(E) and FeLV p15 was combined; 20 μL of this mixture was analyzed (Figure 3). The labeled peaks elute in the same relative positions as those in Figure 2 and the noncoincident peaks retain their relative positions as well. The peak heights in Figure 3 are slightly smaller than expected; this may be due to dilution or sampling errors. Unexpectedly, peak c shows greater fluorescence than predicted based on loading levels. It is not clear why this is so; it may be due to coelution of some decomposition product.

AMV p15. The 15 000 molecular weight protein (24 μg) from avian myeloblastosis virus (AMV p15) was reacted with trypsin, and the resulting peptide mixture was analyzed (Figure 4). Other than the single peak appearing near 120 min, the resultant peptide map bears little similarity to those illustrated in Figure 2. Although mapping is incomplete (acidic peptides are not resolved with this protocol), these data support the contention that no antigenic determinants common to AMV p15 and the 15 000 molecular weight mammalian virus proteins exist.

Comparison of FeLV p30 and MuLV p30. Tryptic peptides from 24 μg of FeLV p30 and 24 μg of MuLV p30 were analyzed in a similar fashion (Figure 5). Peaks labeled k, o, p, and q in the two resultant chromatograms coincide. Coincident peaks at approximately 140 min (ammonia) are discounted as before. Although supposedly equal amounts of protein were analyzed, fluorescence values of peaks in the MuLV p30 map are considerably lower than corresponding peaks in the FeLV p30 map. This may be explained by inherent inaccuracies of the Lowry method for determining protein mass.

As before, tryptic peptides from the two proteins were combined and chromatographed (data not shown). The experiment verified that all labeled peaks were indeed coincident.

[2] The abrupt change and anomalous behavior of the baseline occurring after 600 min result from contaminants present in the first five buffer solutions. These substances, either naturally fluorescent or capable of forming fluorophors after reacting with OPA, are deposited on the resin bed but only partially migrate through it. The high pH borate buffer solution elutes these substances from the column for subsequent fluorescent detection. The extent of the baseline perturbation reflects the amount of contaminants present in the first five buffer solutions and the length of time each was supplied to the resin bed. The small baseline perturbations occurring between 180 and 600 min reflect elution of contaminants less tightly bound to the resin bed than those eluting after 600 min. With elution of each successive buffer solution, some of the contaminants removed from preceding solutions are eluted. The goal of an absolutely flat baseline (and, hence, still higher sensitivity of detection) requires buffer solutions free of such contaminants.

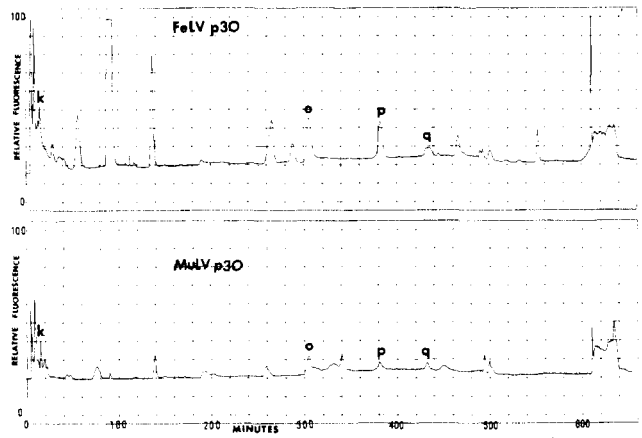

FIGURE 5: Comparison of FeLV p30 and MuLV p30. Tryptic peptides from 24 μg each of the p30 proteins from feline and murine leukemia viruses were chromatographed. Labeled peaks represent coincident peptides thought to originate from regions in the intact protein containing interspecies antigenic determinants. Noncoincident peptides would account for group and type specific antigenic reactivity known to exist. Reduced fluorescent values of murine virus peptides may be explained by errors in the Lowry method of determining protein mass.

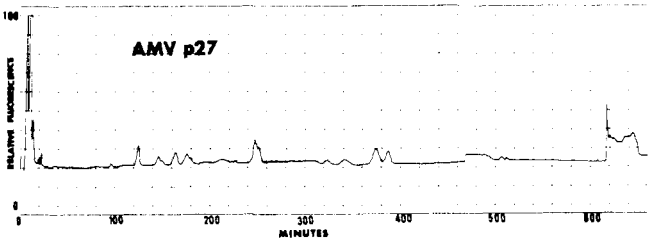

FIGURE 6: AMV p27 tryptic peptides. Tryptic peptides obtained from only 19 μg of the p27 protein from avian myeloblastosis virus were analyzed. There is little or no correspondence between this map and that obtained from the similar molecular weight proteins of mammalian tumor viruses (Figure 5). This supports serological evidence that no common antigenic determinants exist among avian and mammalian tumor virus proteins.

However, shapes of coincident peaks q in the two chromatograms are dissimilar; close inspection of the FeLV p30 map reveals that a second peptide may be eluting nearby. If we omit peak q from consideration because it may be incompletely resolved, we are left only with peaks k, o and p. These have both identical elution positions and similar shapes. Although these proteins are clearly more dissimilar than the p15 proteins, the presence of these three coeluting peaks indicates some regions of sequence identity may exist.

AMV p27. The 27 000 molecular weight protein (19 μg) from avian myeloblastosis virus (AMV p27) was digested with trypsin and the resultant peptide mixture was analyzed (Figure 6). As in the case of AMV p15, the peptide map bears no resemblence to those obtained from the mammalian tumor viruses (Figure 5).

Comparison of MuLV p10 and FeLV p10. Typtic peptides from 18 μg of MuLV p10 and 16 μg of FeLV p10 were analyzed (Figure 7). Labeled peaks in the two chromatograms coincide, verified as before by analyzing a mixture of peptides from the two proteins (data not shown). The off-scale peak at approximately 90 min resulting from deoxycholate present in the FeLV protein masks comparison of the same region in the MuLV chromatogram. The coincident peaks at approximately 140 min represent ammonia. Note that peaks labeled w and x do not display the same peak heights in the two chromatograms, although other coincident peaks have nearly the same peak heights.

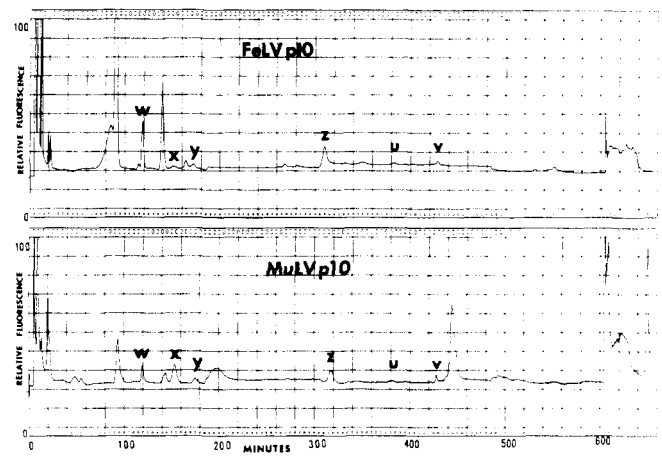

FIGURE 7: Comparison of MuLV p10 and FeLV p10. Tryptic peptides from 18 μg of MuLV p10 and 16 μg of FeLV p10 were chromatographed. At least four peaks (y, z, u, v) coincide and also display similar peak shapes. As in the case of the p15 proteins, this suggests regions of homology exist in the p10 proteins. However, immunochemical tests have not yielded evidence of interspecies antigenic determinants.

Discussion

Conclusions drawn from our results rely upon interpretation of the tryptic peptide maps. Many factors can influence peak shape, such as coelution of different peptides with different fluorescent values or technical inconsistencies. In addition, some peptides exhibit small fluorescence with OPA (Mendez and Gavilanes, 1976). As a consequence of these equivocations, we interpret our data conservatively. We consider as strong evidence of peptide identity only existence of coeluting peaks that also have similar shapes.[3] In addition, very small peaks (such as f and h in Figure 2 and u and v in Figure 7) are considered as coeluting peptides only if they appeared consistantly at their respective positions during replicate analysis.

Schäfer et al. (1975) found that viral p15 polypeptides from different animal species contained mostly group specific antigenic determinants and that the interspecies determinant represented a large proportion of the serological activity of the molecule. Our maps (Figures 2 and 3) demonstrate that the structures of the 15 000 molecular weight proteins from murine and feline leukemia viruses are indeed similar. Peak coincidence and a qualitative evaluation of overall peak shape are taken as evidence that peptides have identical sequences, so the fact that at least five such peptides were found implies that significant regions of homology exist within the two molecules. In contrast, the similar molecular weight protein from avian myeloblastosis virus produced an entirely dissimilar peptide map (Figure 4.) This is consistant with observations that no serological cross-reactivity exists between mammalian and avian viruses (Geering et al., 1970). Thus, our results support data obtained by serological techniques, while yielding structural information at a more detailed level. It is interesting to note that the p15 polypeptide obtained from the murine viral envelope apparently contains regions homologous with the p15

[3] This problem could be resolved if amino acid composition data were available. For example, coeluting peptides from the various proteins could be collected and the amino acid compositions determined. If those compositions were identical, evidence of homology would be greatly enhanced. We are currently attempting to develop the ultramicrotechniques necessary to perform such experiments.

polypeptide taken from the feline viral core. It would be interesting to compare p15 core and envelope polypeptides taken from the same virus.

In contrast to the p15 peptide maps, patterns obtained from the two p30 proteins reveal substantive dissimilarities (Figure 5). However, at least three peptides display both coincidence and similar peak shapes; these regions in the intact protein most likely form the centers for interspecies antigenic determinants, as first suggested by Benson (1974, 1975a). To further verify that our technique provided data consistant with serological studies, we examined tryptic peptides from the major structural protein of avian myeloblastosis virus, AMV p27 (Figure 6). As with p15 proteins, elution profiles were dissimilar. The p30 proteins yielded more than 20 basic tryptic peptide peaks, in general agreement with another column chromatographic method (Buchhagen et al., 1975), a thin-layer mapping technique (Oroszlan et al., 1974), and amino acid composition data of the proteins (Bolognesi et al., 1973). Agreement of our data may be improved after similar analysis of early eluting (acidic) peptides using anion-exchange chromatography.

At least four peptides coincide and display similar peak shapes in the maps from p10 proteins of murine and feline leukemia viruses (Figure 7), indicating that regions of homology exist within those proteins and that interspecies antigenic determinants ought to be present. However, Green et al. (1973) and Schäfer et al. (1975) found only group specific determinants. This discrepancy could be explained if the regions of homology suggested by our data are folded in the protein in such a way that serum antibody has no access, if the determinants are not strong antigens, or if an animal cannot make antibodies against those determinants.

Our discovery of apparent homology in mammalian p10 proteins points out limitations of current serological techniques in protein-structure investigations. Our method also provides advantages not always possible with other methods. For example, the radionuclide-labeling techniques described by Buchhagen et al. (1975) provide high sensitivity but lack resolution because continuous monitoring of effluent peptides cannot be accomplished. Conventional (thin-layer) peptide mapping provides a direct means of determining protein fine structure, but our method allows better quantitation and higher sensitivity. Competitive radioimmunoassay has evolved into a powerful technique for the study of relatedness of proteins, particularly because only nanogram amounts of material are required for most experiments. The taxonomy of tumor viruses proposed by Strand and August (1975) relies heavily on this technique. However, our method provides a comparison of viral evolutionary changes at a more detailed level. As we have shown, weak or absent immunological reactivity is not cause for dismissing protein relatedness.

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Proton Nuclear Magnetic Resonance Study of the Effect of pH on tRNA Structure†

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ABSTRACT: The low-field 220-MHz proton nuclear magnetic resonance (NMR) spectra of four tRNA molecules, *Escherichia coli* $tRNA^{Phe}$, $tRNA_1^{Val}$, and $tRNA^{fMet}_1$, and yeast $tRNA^{Phe}$, at neutral and mildly acidic pH are compared. We find a net increase in the number of resonances contributing to the −9.9-ppm peak (downfield from sodium 4,4-dimethyl-4-silapentanesulfonate) in three of these tRNAs at pH 6, while $tRNA^{fMet}_1$ does not clearly exhibit this behavior. The increase in intensity at this resonance position is half-completed at pH 6.2 in the case of yeast $tRNA^{Phe}$. An alteration at the 5′-phosphate terminus is not involved, since removal of the terminal phosphate does not affect the gain in intensity at −9.9 ppm. Based on a survey of the tertiary interactions in the four molecules, assuming that they possess tertiary structures like that of yeast $tRNA^{Phe}$ at neutral pH, we tentatively attribute this altered resonance in *E. coli* and yeast $tRNA^{Phe}$ to the protonation of the N_3 of the adenine residue at position 9 which results in the stabilization of the tertiary triple A_{23}·U_{12}·A_9. This interpretation is supported by model studies on the low-field proton NMR spectrum of A_N oligomers at acid pH, which reveal an exchanging proton resonance at −9.4 ppm if the chain length $N \geq 6$.

Since their original implication in protein biosynthesis, transfer RNA molecules have been found to function in highly diverse roles within the cell (Littauer and Inouye, 1973). The variety of interactions demonstrated by these molecules in protein synthesis suggests that more than one stable structure may exist in vivo. It would in fact be remarkable to find tRNA molecules binding to aminoacyl-tRNA synthetases, T-factors, codon triplets, and ribosomes without structural alteration. The determination of a detailed structure for yeast $tRNA^{Phe}$ from diffraction analysis of crystalline molecules (review, Sussman and Kim, 1976) has stimulated attempts both to define the precise conformation of tRNA molecules in solution as well as to determine the nature of the conformational changes that arise as a function of the solution conditions. Observed changes in the overall absorbance or circular dichroism properties of individual tRNA molecules as well as unfractionated tRNA mixtures (Riesner et al., 1969; Römer et al., 1970; Goldstein et al., 1972; Cole et al., 1972) indicate that the in vitro structure of tRNA is highly sensitive to ionic strength, divalent and monovalent cations, temperature, pH, and solvent, to a degree not observed in single- or double-stranded RNA structures. In situ conformational probes, such as the 4-thiouridine residue present in certain *Escherichia coli* tRNAs (Delaney et al., 1974) or the Y base in yeast $tRNA^{Phe}$ (Beardsley et al., 1970), reveal similar alterations in conformation, as do the tritium exchange rates of hydrogen-bonded protons in tRNA (Goldstein et al., 1972) and the hydrodynamic properties of the molecule (Fresco et al., 1966; Olson et al., 1976).

The most detailed attempted comparisons of the solution and crystal structure of tRNA have been based upon high-resolution proton nuclear magnetic resonance studies of the hydrogen bonds in a number of specific tRNAs. The key observations that the ring protons of guanine (N_1H) and uracil (N_3H) exchange slowly when they participate in hydrogen-bonded base pairing of tRNA (Englander and Englander, 1972) and that their nuclear magnetic resonance frequency is sufficiently removed from that of the water protons to permit their measurement directly in H_2O (Kearns et al., 1971a) have led to an extensive series of investigations on the solution structure of tRNA (Kearns and Shulman, 1974; Crothers et al., 1974; Daniel and Cohn, 1975; Reid and Robillard, 1975; Robillard et al., 1976). With the recent finding that tertiary as well as secondary hydrogen bond resonances can be detected in the low-field spectral region (Reid et al., 1975), and that the total number of resonances is in fair agreement with that anticipated from the x-ray models (Sussman and Kim, 1976), it seems reasonable to anticipate that a complete assignment of all secondary and tertiary bond resonances will emerge in the near future.

If the conclusion can be accepted that the structure of tRNA in solution, in the presence of sufficient Mg^{2+}, at netural pH and temperature below 37 °C, corresponds reasonably well with the x-ray crystallographic structure of yeast $tRNA^{Phe}$

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