that, in the κ-type proteins, where His-198 is more difficult to protonate, the immunoglobulin fold is more compact than in the case of the λ-type proteins.

X-ray crystallographic studies of the Mcg dimer (Edmundsen et al., 1975) have revealed that the spatial relations between the constant and variable domains in the two light chains are markedly different. By contrast, the two histidines at the identical position in the constant domains of the two light chains as well as those that are observable in the variable domains give identical chemical shifts even at 360 MHz,[5] indicating that, as far as the environment surrounding the histidine residues is concerned, the conformations of constant and variable domains are quite similar for the two monomeric units of the dimer in solution.

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[5] The 360-MHz NMR spectra were measured using a Bruker HXS-360 spectrometer at Stanford Magnetic Resonance Laboratory which is supported by National Institutes of Health Grant RR00711 and National Science Foundation Grant GR 23633.

Shapes of Proteins L1, L9, L25, and L30 from the 50S Subunit of the *Escherichia coli* Ribosome, Determined by Hydrodynamic Studies†

Lallan Giri,* Alfred Franz, and Jan Dijk

ABSTRACT: Proteins L1, L9, L25, and L30, purified by a nondenaturing method from the 50S ribosomal subunit of *Escherichia coli* A19, have been characterized. The four proteins were studied under conditions which resemble those used for reconstitution experiments. These proteins have $s^0_{20,w}$ values of 2.0 S, 1.8 S, 1.8 S, and 1.0 S and $D_{20,w}$ values of 8.4 × 10^{-7}, 9.0 × 10^{-7}, 14.0 × 10^{-7}, and 15.0 × 10^{-7} cm^2/s. Apparent specific volumes at 20 °C are 0.738, 0.733, 0.700, and 0.735 mL/g for the four proteins. The respective molecular weights determined by sedimentation equilibrium are 25 000, 17 300, 12 000, and 6500. The intrinsic viscosity values for the four proteins are 4.0, 5.5, 3.6, and 3.2 mL/g. From these hydrodynamic parameters L1 and L9 appear to have globular or at most only slightly elongated shapes, whereas L25 and L30 appear to be definitely globular.

Many innovative studies have been reported in the literature in an attempt to understand the structure and function of ribosomes. For better understanding of protein–protein and protein–RNA interactions in ribosomes, the shapes of several ribosomal proteins have been determined in situ, on the surface of the ribosome, and in solution. The elucidation of a number of specific antibody binding sites for individual proteins on the ribosome using immune electron microscopy techniques has given evidence that some of the protein molecules may have

† From the Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, D-1000 Berlin 33 (Dahlem), Germany. *Received December 14, 1978.*

* Present address: Department of Biochemistry, University of Maryland, School of Medicine, Baltimore, MD 21201.

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elongated shapes [reviewed in Stöffler & Wittmann (1977)].

Hydrodynamic studies have been performed on proteins S1, S3, S4, S5, S7, S8, S15, S17, S18, and S20 (Rhode et al., 1975; Paradies & Franz, 1976; Giri et al., 1977; Laughrea & Moore, 1977; Giri & Subramanian, 1977; Giri & Franz, 1978; Georgalis & Giri, 1978; Franz et al., 1979) and proteins L3, L6, L7/L12, L11, and L24 (Wong & Paradies, 1974; Giri et al., 1977, 1978; Giri & Dijk, 1979). By these studies, both elongated and globular shapes have been found for *Escherichia coli* ribosomal proteins.

Very little is known about the functional role of proteins L1, L9, L25, and L30. Protein L1 binds to the 23S RNA (Garrett et al., 1974) and protects 148 nucleotides at the 3′ terminal segment of the 23S RNA from the nuclease digestion (Spierer et al., 1975; Branlant et al., 1976a). Protein L25 binds to both 5S RNA (Horne & Erdmann, 1972; Gray et al., 1973) and 23S RNA (Branlant et al., 1976b).

By immune electron microscopy three antibody binding sites for protein L1 and single antibody binding sites for L9, L25, and L30 have been found. The three antibody binding sites for L1 are remote from each other, indicating an elongated shape in situ [reviewed in Brimacombe et al. (1978)]. From a small-angle X-ray scattering study an elongated shape for protein L25 has been inferred (Österberg et al., 1976).

Until now no physical studies have been made on proteins L1, L9, and L30 in solution. In this study, in order to characterize physically the four proteins prepared by a nondenaturing purification procedure (Dijk & Littlechild, 1978), we have determined several physical parameters such as the molecular weight, diffusion coefficient, partial specific volume, intrinsic viscosity, and sedimentation coefficient. These parameters have been used to determine the probable size and shape of these proteins. The results of this study suggest a globular shape for proteins L25 and L30 and a slightly asymmetric shape for L1 and L9.

Materials and Methods

(*a*) *Protein Preparation.* The four proteins were prepared by an improved purification procedure (J. Dijk and I. Ackermann, unpublished experiments). Proteins L9, L25, and L30 were obtained by extraction of the 50S subunit with 2 M NaCl in the presence of 0.01 M Mg^{2+} at pH 7.0 and fractioned on CM-Sephadex C-25 with a NaCl gradient in 0.01 M sodium phosphate, pH 7.0, followed by gel filtration on Sephadex G-75 superfine. Protein L1 was obtained by extracting the subunit with 2 M LiCl in the presence of 0.01 M EDTA at pH 7.0. It was purified by the same chromatographic methods as described above. The proteins were concentrated by adsorption–desorption on small CM-Sephadex columns. They were characterized and tested for purity by two-dimensional gel electrophoresis (Kaltschmidt & Wittmann, 1970), by one-dimensional gel electrophoresis in the presence of $NaDodSO_4$ (Laemmli & Favre, 1973), and by amino acid analysis.

Protein samples were exhaustively dialyzed in SpectraPor 3 dialysis tubing against 0.35 M NaCl, 0.05 M sodium phosphate, pH 7.0, containing in addition 1 mM dithioerythritol, 0.1 mM phenylmethanesulfonyl fluoride, and 0.1 mM benzamidine hydrochloride. The samples and the buffer were filtered through Nucleopore membranes with a pore size of 0.4 μm.

(*b*) *Amino Acid Analysis.* Aliquots of protein solutions were subjected to hydrolysis in 6 N hydrochloric acid containing 0.2% 2-mercaptoethanol under vacuum at 110 °C for 24 h, followed by amino acid analysis on a Durrum D-500 analyzer. Norleucine was used as an internal standard.

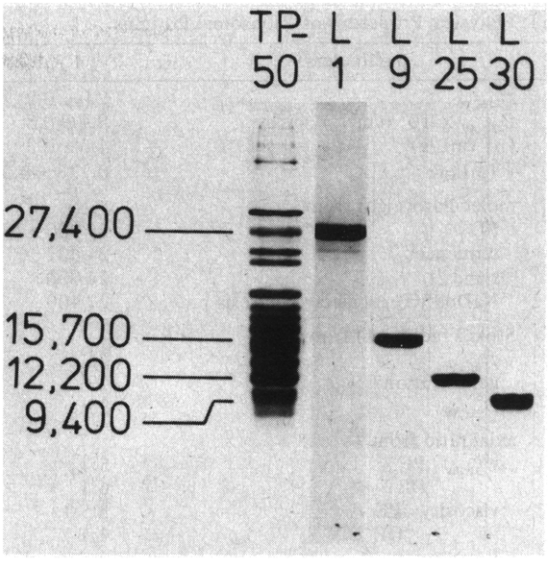

FIGURE 1: Gel electrophoresis of proteins L1, L9, L25, and L30 in $NaDodSO_4$ containing slab gels. Approximately 5 μg of protein sample was applied to each slot. As a comparison the separation of the total mixture of proteins from the 50S subunit (TP-50) is also shown. The molecular weight, assigned to each band by comparison with marker proteins, is indicated at the left. Electrophoresis was from top to bottom.

(*c*) *Concentration Determination.* The concentrations of protein solutions were determined by amino acid analysis as described above and from nitrogen assays (Jaenicke, 1974) by using ammonium sulfate as a standard. The nitrogen contents of the proteins were calculated from their amino acid sequence and composition data. Good agreement was found between the two methods.

(*d*) *Hydrodynamic Measurements.* The viscosities, apparent specific volumes, sedimentation coefficients, diffusion coefficients, and molecular weights by sedimentation equilibrium were determined as described in our earlier publication (Giri et al., 1978; Giri & Dijk, 1979).

Results

(*a*) *Protein Solubility, Gel Filtration, and Sodium Dodecyl Sulfate Gel Electrophoresis.* Proteins L1, L9, L25, and L30, purified under nondenaturing conditions, were checked for purity and possible proteolytic degradation by both two-dimensional gel electrophoresis and one-dimensional gel electrophoresis in the presence of $NaDodSO_4$. Protein L1 contained traces of L3 and L6, and protein L25 was contaminated with a trace of L29. Since by densitometry of the gel it was found that the contamination level was below 2%, it was considered negligible. Proteins L9 and L30 showed no contamination (Figure 1). The molecular weights, obtained by comparison with marker proteins on the $NaDodSO_4$ gels, are also indicated in Figure 1.

The solubility of these four proteins under the conditions used here was excellent. The solubility of protein L1 is very dependent on the presence of phosphate buffer; in Hepes buffer of the same pH and ionic strength its solubility is limited to 2 mg/mL. Protein concentrations of 6 mg/mL for L1 and 8–10 mg/mL for the other proteins have been attained without visible precipitation. However, as the experiments described in this paper indicate, some protein aggregation usually occurred at high concentrations.

All proteins were subjected to analytical gel filtration on Sephadex G-50 and G-75 (superfine) columns in the NaCl–phosphate buffer used for the other experiments. At protein concentrations of 1–2 mg/mL all four proteins gave

Table I: Physical Properties of Ribosomal Proteins

parameters[a]	L1	L9	L25	L30
$s^0_{20,w}$	2.0 ± 0.2	1.8 ± 0.15	1.8 ± 0.1	1.0 ± 0.1
$D_{20,w} \times 10^7$ (cm²/s)	8.4 ± 0.8	9.0 ± 1.0	14.0 ± 0.9	15.0 ± 1
$[\eta]$ (mL/g)	4.0 ± 0.3	5.5 ± 0.3	3.6 ± 0.2	3.2 ± 0.1
$\bar{v}$ (mL/g)	0.738 ± 0.003	0.733 ± 0.007	0.700 ± 0.005	0.735 ± 0.005
molecular weight from				
SE	25 000 ± 500	17 300 ± 300	12 000 ± 500	6500 ± 500
sequence[b]	24 602		10 695	6410
s and D	24 000	19 000	11 000	6600
$NaDodSO_4$ gel electrophoresis	27 400	15 700	12 200	9400
Stokes radius (Å) from				
$s^0_{20,w}$	26	20	15	13
gel filtration	23	22	13	11
$D_{20,w}$	25	23	15	14
axial ratio from				
$s^0_{20,w}$ PE	5:1	4:1	4:1	3.5:1
OE	6:1	4.5:1	4.5:1	4:1
viscosity PE	3.5:1	4.5:1	3:1	2.5:1
OE	4:1	6:1	3.5:1	2.5:1
frictional ratios from $s^0_{20,w}$	1.30	1.20	1.15	1.17
approximate radius of gyration (Å)	20-25	15-20	12	10

[a] SE = sedimentation equilibrium; PE = prolate ellipsoid; OE = oblate ellipsoid. [b] Values taken from literature.

a single, sharp, and symmetric peak. The elution positions were compared with those of proteins with known Stokes radii. Stokes radii of 23 Å for L1, 22 Å for L9, 13 Å for L25, and 11 Å for L30 were found (average from four independent experiments on both Sephadex G-50 and G-75).

(*b*) *Apparent Specific Volume.* In order to accurately evaluate the sedimentation coefficients and molecular weights of the four proteins, the apparent specific volumes (ϕ^*) at constant chemical potential were determined from the experimentally measured density data.

The measured densities of all the four proteins were linear functions of protein concentration. The density of L9 as a function of protein concentration is presented in Figure 2. Occasionally, at concentrations over 1.5 mg/mL some deviation from the linearity was observed. This was more pronounced for L1 and L9 than for L25 and L30. The apparent specific volume values were found to be independent of protein concentration. The limiting apparent specific volume value of each protein was used as partial specific volume $\bar{v}$ in $s_{20,w}$ and molecular weight determination. The limiting values of apparent specific volume which were assumed to be similar to partial specific volume are given in Table I. The experimentally determined apparent specific volume values of L1, L9, and L30 are somewhat smaller than the $\bar{v}$ values estimated from amino acid composition whereas L25 gave a comparable value. The values of apparent specific volume estimated from amino acid composition of L1, L9, L25, and L30 were 0.746_9, 0.741_8, 0.706_3, and 0.750_5, respectively.

(*c*) *Sedimentation Velocity.* The apparent sedimentation coefficients of proteins L1, L9, L25, and L30 were measured at several protein concentrations. A single broad symmetric boundary was observed for each protein, except L30 which showed a somewhat broader asymmetric boundary than the remaining proteins. The dependence of the $s^0_{20,w}$ values on the concentration is shown in Figure 3. These plots give $s^0_{20,w}$ values of 2.0 S, 1.8 S, 1.8 S, and 1.0 S for proteins L1, L9, L25, and L30, respectively. Proteins L1 and L9 showed a concentration dependence with a positive slope (Figure 3A). This effect can be attributed to aggregation. Protein L25 showed a concentration dependence of the usual type with a negative slope, whereas very little or no dependence was observed for L30.

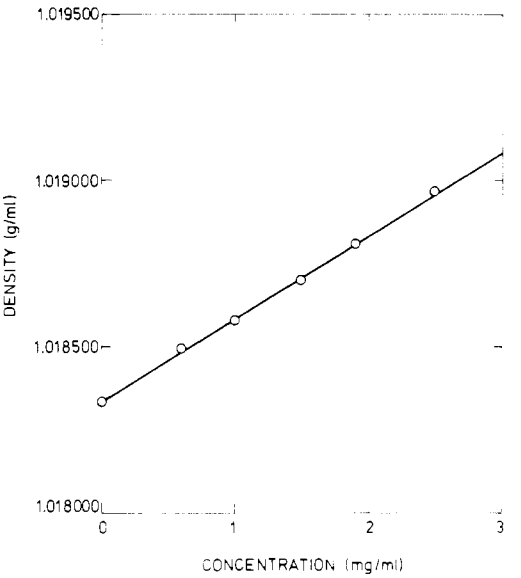

FIGURE 2: Experimentally measured densities of protein L9 as a function of concentration. Samples were dialyzed to an equilibrium before the measurements. The measurements were carried out in a precision digital densitometer (DMA 60 and DMA 601M, Paar KG, Graz) at 20 °C.

(*d*) *Diffusion Coefficients.* The diffusion coefficients were determined by using a synthetic boundary centerpiece in the analytical ultracentrifuge. At low protein concentrations an accurate integration of the boundary area was not possible. The calculated apparent diffusion coefficient values from these boundaries showed a 10–12% variation in two sets of experiments. The apparent $D_{20,w}$ values determined at three to four different concentrations of each protein were averaged (Table I). The Stokes radii calculated from the $D_{20,w}$ of all the four proteins are in good agreement with those inferred from gel filtration (Table I).

(*e*) *Molecular Weight.* The apparent weight-average molecular weights were determined from the average slopes of the least-squares fit of ln c vs. r^2 in several short column runs. The apparent weight-average molecular weight values obtained at protein concentrations up to 1.6 mg/mL were extrapolated to zero concentration in order to obtain true

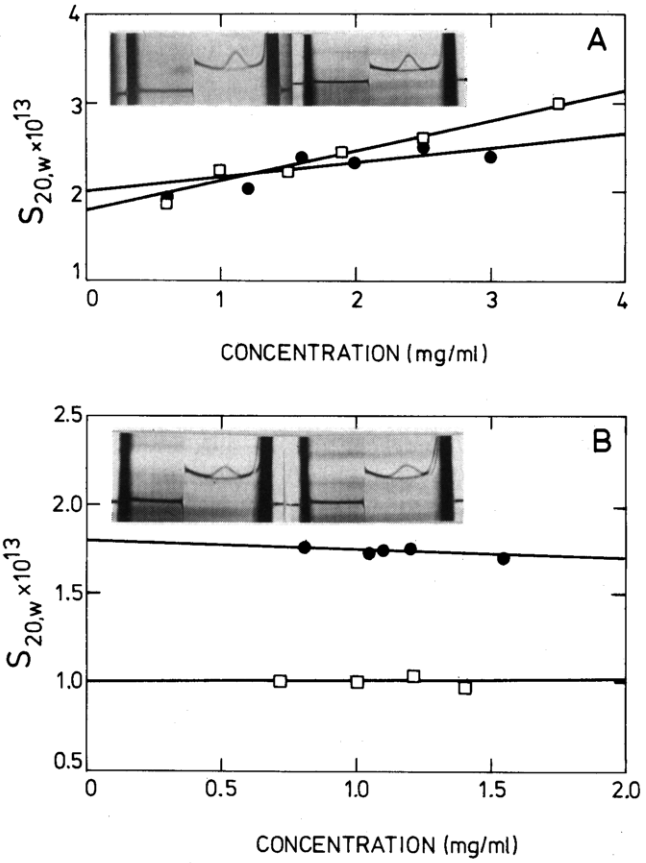

FIGURE 3: Sedimentation velocity. (A) The apparent $s_{20,w}$ of L1 (●) and L9 (□) as a function of concentration. Inset: Schlieren pattern of L1 (left) and L9 (right). (B) The apparent $s_{20,w}$ of L25 (●) and L30 (□) as a function of concentration. Inset: Schlieren pattern of L25 (left) and L30 (right). A capillary type synthetic boundary centerpiece was used at a speed of 56000 rpm at 20 °C. These pictures were taken 20 min after the rotor reached maximal speed. The Schlieren pattern of the four proteins shown in the figure represents sedimentation experiments performed at approximately 1.5 mg/mL concentration.

molecular weights. Up to this concentration range these proteins showed a linear plot of ln c vs. radius squared, and the plot of apparent molecular weights vs. protein concentration showed very little or no concentration dependence. The use of considerably low concentration of protein enabled us to avoid aggregation, and true molecular weights with less degree of uncertainty (2–8%) were obtained.

(*f*) *Viscosity.* The reduced viscosity was determined for all the four proteins, and by extrapolation to zero protein concentration, the intrinsic viscosity [η] values were found to be 4.0, 5.5, 3.6, and 3.2 mL/g for proteins L1, L9, L25, and L30, respectively. The plots of η_{sp}/c vs. protein concentration for the four proteins are shown in Figure 4. The reduced viscosities of L1 and L9 showed some concentration dependence which was more pronounced with L9 than with L1. Nearly no concentration dependence was observed for L25 and L30. The intrinsic viscosity values found for L1, L25, and L30 are not very different from those reported for some globular proteins (2.5–3.5 mL/g). On the other hand, the value for L9 is somewhat higher (Tanford, 1961; Yang, 1961).

Shape factors of 3.8, 5.3, 3.6, and 3.1 were calculated for L1, L9, L25, and L30, respectively, by assuming 30% hydration. These values would correspond to approximate axial ratios of 3.5, 4.5, 3, and 2.5 for the four proteins from Perrin's function, by assuming a prolate ellipsoidal model. For an oblate ellipsoidal model the axial ratios would be 4, 6, 3.5, and 2.5 for these proteins.

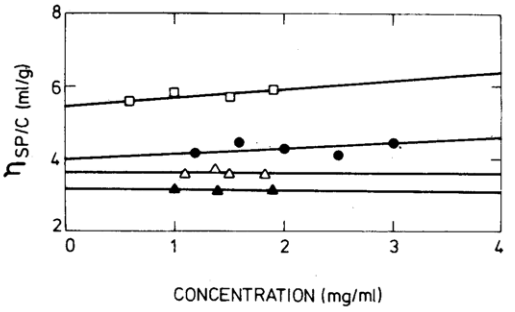

FIGURE 4: Reduced viscosity of L1 (●), L9 (□), L25 (△), and L30 (▲) as a function of concentration.

(*g*) *Stokes Radius and Frictional Ratios.* Stokes radii of the four proteins were deterimined from gel filtration and from the experimentally determined $s^0_{20,w}$ values by using the equations described earlier (Tanford et al., 1974; Giri et al., 1978). The latter method gave Stokes radii of 26, 20, 15, and 13 Å which were comparable to the values obtained from the gel filtration. Stokes radii were also calculated from diffusion coefficients of the four proteins and were found to be in good agreement with those obtained from gel filtration and $s^0_{20,w}$ (Table I).

Frictional ratios were determined from the $s^0_{20,w}$, $\bar{v}$, and molecular weight data by using the appropriate equation (Rhode et al., 1975; Giri et al., 1978) and from intrinsic viscosity. The values are given in Table I. The frictional ratios f/f_0, estimated from viscosity by using Perrin's function, are somewhat smaller than those obtained from $s^0_{20,w}$. In order to determine the overall shape of proteins, we used the f/f_0 values obtained from $s^0_{20,w}$. Sedimentation coefficients and intrinsic viscosities as low as these are difficult to measure, especially at low concentrations. However, the individual values in two experiments were reproducible with a random error of 5–10%. A 10% uncertainity in $s^0_{20,w}$ determination would cause approximately 8–9% error in frictional ratio estimation whereas a leverage of lesser extent would be caused by similar uncertainity in intrinsic viscosity values.

The approximate radii of gyration for the four proteins were calculated by using the appropriate equations (Pilz, 1973) for sphere (L25 and L30) and oblate ellipsoids (L1 and L9) with semiaxes a, b, and c ($b = c$). The semiaxes were estimated by using the axial ratio and dry volume. The radii of gyration in this study are only approximations. These can be experimentally and accurately determined by small-angle X-ray diffriction at very high protein concentration.

Discussion

In this study physical parameters and the general shapes of the four hitherto uncharacterized proteins were determined. These proteins were prepared by a gentle procedure avoiding the use of urea and other denaturing agents. Nuclear magnetic resonance studies of ribosomal proteins, prepared under both nondenaturing and denaturing conditions, have shown that the former proteins contain substantially more tertiary structure (Morrison et al., 1977). Furthermore, the nondenatured proteins were more soluble at high ionic strength.

Under present experimental conditions, which are similar to the reconstitution conditions, proteins L1, L9, L25, and L30 behave as monomeric molecules at low protein concentrations with molecular weights of 25000, 17300, 12000, and 6500, respectively, as determined by sedimentation equilibrium experiments. The molecular weight of L1 obtained in this study is somewhat higher than that reported earlier from a similar method (Dzionara et al., 1970), and it is in excellent

agreement with that obtained from amino acid sequence data, namely, 24 602 (Brauer & Öchsner, 1978). The molecular weight of L9 in this study is in very good agreement with that reported earlier (Mora et al., 1971). Protein L25 shows a molecular weight which is somewhat higher than that obtained from amino acid sequence, namely, 10 694 (Dovgas et al., 1975; Bitar & Wittmann-Liebold, 1975). The molecular weight for L30 obtained in this study is in excellent agreement with that reported from amino acid sequence data, namely, 6411 (Ritter & Wittmann-Liebold, 1975).

From polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$, we have obtained molecular weight values for L1, L9, L25, and L30 of 27 400, 15 700, 12 200, and 9400, respectively. By using a similar technique others have obtained similar values, i.e., 26 700, 17 300, 12 000, and 11 200 (Dzionara et al., 1970) and 27 600, 16 900, 9800, and 8800 (Zimmermann & Stöffler, 1976). However, the values obtained from this technique should be compared with caution because it is known that the $NaDodSO_4$ electrophoresis gives somewhat high molecular weights with small basic proteins and also shows variations in the results when different buffer systems are used.

Globular proteins of molecular weights similar to L1, L9, L25, and L30 would be expected to have $s_{20,w}$ values of 2.7 S, 2.1 S, 2.0 S, and 1.1 S, respectively. If we assume 30% hydration, the observed $s_{20,w}$ values are compatible with a globular shape for all of the four proteins.

Using the viscosity data and assuming 30% hydration, we obtained a slightly higher axial ratio (4.5–5.0:1) for protein L9 and slightly smaller axial ratios for proteins L1, L25, and L30 than the ratios estimated from $s^0_{20,w}$.

The interpretation of the general shape of protein molecules depends very much on the definition of the limiting axial ratio. If one considers an axial ratio of 4–5 or a frictional ratio of 1.3, the maximum limit for a globular protein molecule, L1 and L9 can be classified as globular proteins; otherwise, they may be regarded as slightly asymmetric or elongated molecules. Stokes radii of L1, L25, and L30 are comparable to those of globular proteins of similar molecular weights whereas the Stokes radius of L9 indicates a more asymmetric shape for this protein.

From immune electron microscopy [reviewed in Stöffler & Wittmann (1977)] and from small-angle X-ray scattering (Österberg et al., 1976), elongated shapes have been inferred for proteins L1 and L25. On the other hand, protein L1, though it may be slightly asymmetric, does not appear to be as elongated from our study as it has been suggested to be in situ. Similarly, our results for L25 differ from those of small-angle X-ray scattering studies (Österberg et al., 1976) which were peformed on a protein L25 that was isolated under denaturing conditions. Such proteins have been found to contain less tertiary structure (Morrison et al., 1977), and this could account for the observed differences.

It is important to keep in mind that dimensions of proteins described in this study are only approximations assuming equivalent ellipsoidal models. These should be interpreted cautiously as giving accurate dimensions of real particles.

Proteins L1 and L25 protect a substantial part of 23S RNA and 5S RNA against nuclease digestion (Branlant et al., 1976a,b; Gray et al., 1973), and one might conclude an elongated shape for L1 and L25. However, this conclusion should be considered with caution since the mutual protection against enzymes could arise, to a large extent, from the secondary and tertiary structure of the components themselves.

In conclusion, we propose a globular or slightly elongated shape for proteins L1 and L9 and a definite globular shape for L25 and L30.

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Membrane Glycopeptides from Virus-Transformed Hamster Fibroblasts and the Normal Counterpart†

Mary Catherine Glick

ABSTRACT: Comparisons of membrane glycopeptides from baby hamster kidney fibroblasts (BHK_{21}/C_{13}) and a clone transformed by Rous sarcoma virus (C_{13}/B_4) were made by using cells metabolically labeled with radioactive D-glucose and L-fucose. Most of the glycopeptides were metabolically labeled with both the general and the specific glycoprotein precursors. The glycopeptides obtained from the cell surface by controlled trypsinization were representative of the surface membrane as shown by comparing them with those of purified membrane preparations. The trypsin-removable glycopeptides from both cell types were further processed and examined by successive chromatography on Sephadex G-50 and DEAE-cellulose. The chromatographic distribution patterns showed that each cell type had glycopeptides of similar characteristics, although the proportions of the glycopeptides differed dramatically between the two cell types. After transformation there was an increase in the larger, more highly charged glycopeptides. This was verified by the increased sialic acid content in these glycopeptides. Some of the glycopeptides were homogeneous after the size and charge separations, since a variety of procedures did not separate them further. The apparent homogeneity and reasonably few species obtained may be due to the methods of isolation, with the procedures selecting particular glycopeptides from the external portion of the membrane. These results corroborate the concept and show for the first time that virus transformation is accompanied by an increase in certain species of glycopeptides rather than de novo synthesis.

Membrane glycoproteins from virus-transformed and tumor cells differ from those of the normal counterparts. This has been shown in many systems by using a variety of methods. Among the direct methods used have been polyacrylamide gel electrophoresis of purified surface membranes, using metabolic labeling or Coomassie blue staining for the detection of the proteins and glycoproteins, or whole-cell preparations after surface labeling by different techniques. Others have examined the glycopeptides obtained after protease digestions of a variety of cell types (Fishman & Brady, 1976; Glick, 1976a; Emmelot et al., 1977).

The glycopeptides which were removed from the surface of virus-transformed cells with controlled trypsinization were shown, after subsequent Pronase digestion, to be different from those of the norman counterparts by the criterion of the elution patterns from Sephadex G-50 columns (Glick, 1974a; Warren et al., 1978). These observations were extended to show that the appearance of particular glycopeptides was directly correlated with tumorigenesis (Glick et al., 1974) and that human tumors (Glick, 1976b), including leukemias (Van Beek et al., 1975), showed similar alterations. In fact, the altered surface glycopeptides have been reported to be the most consistent change accompanying malignancy (Van Beek et al., 1977) and have been reviewed (Warren et al., 1978). It must be remembered that these glycopeptides were derived from glycoproteins on the external side of the membrane and indeed, for this very reason, are extremely important in defining a tumor cell.

In spite of the many cell types which have been reported to show these altered glycopeptides [see Warren et al. (1978) for a review], no data have been reported to determine the unique or ubiquitous nature of the glycopeptides. Are the glycopeptide alterations quantitative or qualitative? In order to answer this question, one must examine the heterogeneity of the glycopeptides. Only a brief report (Glick, 1974a) showed that the glycopeptides can be further separated on DEAE-cellulose and another showed their behavior on Con A–Sepharose (Ogata et al., 1976). The studies reported here suggest that the latter separation on Con A–Sepharose was not sufficient and must yield a mixture of glycopeptides. Moreover, the separation on Sephadex G-50, followed by DEAE-cellulose chromatography, revealed a spectrum of glycopeptides.

The glycopeptides were removed by trypsin from the surface of transformed hamster cells (C_{13}/B_4) and the normal counterpart (BHK_{21}/C_{13}). Subsequent digestion of the surface glycopeptides with Pronase as well as further processing left species of glycopeptides representative of the surface of both cell types and showing clearly the alterations observed originally after virus transformation (Buck et al., 1970). As a result of further purification of these glycopeptides, evidence is presented here that all species appeared in both cell types, although the percentages were vastly different. In addition, although there was some heterogeneity of size and charge, the species of glycopeptides were amazingly few. It has thus been possible to isolate some of these glycopeptides in homogeneous

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