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A Study of the Parameters Which Determine the Conformation of Linear Polypeptides in Solution by Synthesis of Models and Determination of Thin Film Dialysis Rates†

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ABSTRACT: A series of hexapeptides with maximum conformational flexibility have been synthesized and studied by the technique of thin film dialysis. The results indicate that the conformation may not be completely random in a given solvent environment and temperature although a degree of randomness has not been excluded. The major contribution to the conformation, within the restrictions expected from conformational mapping, has been shown to be the solvent effect. Electrostatic effects between charged groups were shown to be of little importance when 0.1 M sodium chloride is the solvent. The most extended conformation resulted when the terminal amino acid residues were highly polar.

Recently because of the rapid advances made in high-resolution nuclear magnetic resonance and attempts to calculate minimal energies by computer, considerable attention is being directed toward the conformation in solution of all types of polypeptides (Deber *et al.*, 1969). Such studies are progressing from the simpler problem of the interpretation of results with the rigid cyclic representatives to the more difficult problem of reliable interpretation with the linear ones.

Linear polypeptides without stabilizing covalent cross-linkages have in the past generally been referred to as "random coils." Nonetheless extensive studies (Craig and Harfenist, 1963; Craig, 1967; Craig *et al.*, 1971) by thin film dialysis have shown that in favorable solvent environments such peptides give straight line escape plots indicative of a single diffusional size or at least a narrow distribution of sizes or conformations too similar to be detected by a deviation from linearity. However, the rate of dialysis was found to be highly dependent on the solvent environment and could easily be altered by a small change in pH, ionic strength, or addition of a low concentration of alcohol, urea, guanidinium chloride, or formamide. On the other hand, the rates of dialysis of rigid covalently bound polypeptides such as bacitracin or gramicidin SA were not influenced this way or influenced to a much less extent. It was concluded that in the case of the linear polypeptides much less conformational stability was the reason for the difference.

Simple dipeptides were of special interest in the general study because the conformations of the side chains rather than that of the peptide backbone would be expected to have the greatest influence on the dialysis rate if the way the side chains are folded is determined by the particular solvent environment. The data obtained (Burachik *et al.*, 1970) gave strong support to this theory but nonetheless there were a number of ambiguities in the interpretation. It seemed apparent that the hydrophobic interactions of the side chains were important and that electrostatic interaction played an important role where the side chains contained charged groups of like sign. A later study with lysylglutamic acid or glutamyllysine did not support the theory of a primary effect of electrostatic interaction of the side chains.

In order to ascertain if the same parameters which influence the interactions of the side chains in dipeptides and their conformations would also be those influencing the conformations of longer peptides it seemed of interest to synthesize a series of hexapeptides, X-Gly_4-Y, in which the same amino acids used in the dipeptide study were separated by four glycine residues and to study their rates of dialysis by thin film dialysis in different solvent environments. Glycine peptides theoretically and by dialysis (Burachik *et al.*, 1970) are known to have the least restrictions on their "allowed" (Ramachandran and Sasisekharan, 1968) conformations. This paper reports such a study. It also reports data with two further series of hexapeptides where the two terminal peptide residues are connected by alanine and proline residues. The latter would be expected to have the most rigid conformation and to be the most extended.

The X-Ala_4-Y peptides would be expected to be less compact than the X-Gly_4-Y series but more compact than the X-Pro_4-Y series. Polyproline peptides have not been studied previously by thin film dialysis. With these peptides there exists the opportunity of either cis or trans forms but with a series of adjacent proline residues solvent conditions will usually favor the all-cis form with a right-handed compact helix or all trans forms in a left-handed extended helix (Strassmair *et al.*, 1969). The extended peptide with the trans arrangement of proline residues would be expected to be the most stable form in water.

Experimental Section

The peptides in this study were prepared by the Merrifield solid phase method (Stewart and Young, 1969) from protected

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Boc amino acids. The *O*-benzyl derivatives of Boc tyrosine and threonine, the ε-carbobenzoxy derivative of Boc lysine, the nitro derivative of Boc arginine, and the γ-benzyl ester of Boc glutamic acid were employed to ensure the protection of the side chains of the functional amino acids.

The protected Boc amino acids were purchased from Protein Research Institute, Osaka, Japan. In the case of incorporation of radioactive amino acids Boc-[^{3}H]Gly was obtained commercially from New England Nuclear, Boston, Mass., and Boc-[^{14}C]Ala was prepared from L-[^{14}C]Ala (New England Nuclear) by the procedure of Schwyzer (Schwyzer *et al.*, 1959). For maximum incorportation the radioactive derivatives were diluted and an equivalent of derivative was applied to the resin prior to coupling with the normal amount of cold derivative.

The first amino acid derivative was coupled to the chloromethylpolystyrene support (Bio beads from Bio-Rad, S-xl, 200–400 mesh) in the presence of triethylamine and refluxing ethanol. Reaction was normally allowed for 24 hr after which a degree of substitution of *ca.* 0.5 molar equiv of Boc amino acid per gram of resin had been attained.

The usual procedure of peptide synthesis was followed in which the Boc group was removed by 30% trifluoroacetic acid in dichloromethane for 30 min after each coupling. A threefold excess of Boc amino acid was applied for each coupling step in dichloromethane and a fivefold excess for coupling steps in combinations of dimethylformamide and dichloromethane. Frequently a batch of resin was treated up to the pentapeptide stage, then divided into separate portions and each coupled to different Boc amino acids. At the completion the peptides were separately cleaved from the resin with hydrogen bromide bubbled through a suspension of the resin in anhydrous trifluoroacetic acid. This gave a yield of several analogous hexapeptides. They were purified by one of the following procedures: Sephadex (SE G-25 or DEAE A-25), using pH gradients of ammonium acetate–acetic acid and ammonium acetate–ammonia, preparative paper electrophoresis, or countercurrent distribution. The purified peptides in ammonium acetate buffer at different pH's were brought to a pH of *ca.* 5.0 with ammonium hydroxide or acetic acid. The volatile salts were eliminated by two successive lyophilizations.

For the synthesis of arginyl peptides the nitroarginyl derivative was obtained from the resin after cleavage in hydrogen bromide–trifluoroacetic acid. It was then hydrogenated using a catalyst of palladium on barium sulfate in the presence of 1 atm of hydrogen. By interruption a crude mixture of incompletely hydrogenated products could be obtained, which were chromatographed on SE G-25 Sephadex. In this way NO_2Arg-Gly$_4$-NO_2Arg, NO_2Arg-Gly$_4$-Arg, and Arg-Gly$_4$-NO_2Arg and the analogous alanyl$_4$ peptides were separated from each other. The dinitroarginyl peptides emerged using a gradient of 0.1 N acetic acid to 0.1 N ammonium acetate, the mononitroarginyl peptides emerged during the continued elution with 0.1 N ammonium acetate. They were clearly resolved as doublet peaks in which the first peak was characterized as NO_2Arg-Gly$_4$-Arg and the latter as Arg-Gly$_4$-NO_2Arg by treatment with carboxypeptidase B. The enzymatic digest was examined by amino acid analysis and paper electrophoresis showing that the C-terminal arginyl residue of NO_2Arg-Gly$_4$-Arg was cleaved whereas it was left intact in Arg-Gly$_4$-NO_2Arg. Derivatization of the peptides to give the methyl ester and to give the *N*-acetyl derivative was achieved using the methods of Coulter and Hann (1968, 1971).

The membranes employed were derived from cellulose, 18/32 Visking. These were glycineamidated (Chen *et al.*, 1972) for 24 hr at room temperature or for 4 hr at 65° and acetylated (Craig and Konigsberg, 1961) in 33% acetic anhydride in anhydrous pyridine at room temperature for 3–5 hr. There was only a gradual reduction in the porosity of the membrane after the 3-hr period.

The degree of acetylation of the membranes, treated for 5 hr in acetic anhydride and pyridine, was determined at *ca.* 0.5 mol/mol of total glucose by cleavage with methanolic hydrogen chloride and estimation of liberated methyl acetate by the hydrazine reagent (Hestrin, 1949). This indicates $^{1}/_{6}$ of total substitution assuming there are 3 equiv of hydroxyl groups per residue of glucose.

By an alternative procedure the membrane was longitudinally stretched (Craig and Konigsberg, 1961) by 30% prior to chemical modification. An acetylation time of 1 hr was now required to reduce the permeability to a level equivalent to the 5-hr acetylated unstretched membrane. Despite an assumed difference in the degree of acetylation of stretched and unstretched membranes they gave very similar permeability properties through the range of peptides examined. The membranes were incorporated into an apparatus previously described (Craig and Konigsberg, 1961) in which the inner membrane volume was about 0.5 ml, the outer volume about 3.5 ml.

Additional work was performed on nonacetylated membranes that had just been longitudinally stretched. Close cross comparisons of dialysis data in the acetylated and nonacetylated membranes could not be made as the nonacetylated membranes were considerably more porous than the acetylated membranes.

The membranes were calibrated during usage at regular intervals with Tyr-Gly$_4$-Tyr in 0.01 N acetic acid. As the membranes generally undergo a slow change and become somewhat more porous with repeated use it was necessary to make appropriate corrections to the dialysis time. Providing the half-escape time of Tyr-Gly$_4$-Tyr varied by no more than ±30% from its original value, multiplication by an appropriate ratio was found to give reliable comparative values for the escape time of all of the peptides under study.

For comparative dialysis *ca.* 1 mg (1.5 μmol) of hexapeptide was usually employed in the 0.5 ml of retentate solution. These were dialyzed in 0.1 M NaCl at unadjusted pH (*i.e.*, *ca.* pH 5.0) or in 0.1 M NaCl at pH 3.5 (adjusted by adding dilute hydrochloric acid to the solution). At pH 3.5 the hydrochloric acid is about 0.3 mM and there is about 1.0 μmol of acid/3.5 ml of outer dialysis solution. Exchange of the outer solution one or two times normally ensures the complete adjustment to pH 3.5 of the 1.5 μmol of peptide on the interior of the membrane and subsequent dialysis occurs at that pH. For more accurate control of pH, half-escape times were measured in 0.1 M sodium chloride in a background of 0.01 M buffer (formate, acetate, or phosphate, depending on the pH examined).

Rates of emergence of the peptides from the dialysis membrane were followed by absorbance at 225 nm, or where radioactive amino acids had been incorporated in the peptide, by liquid scintillation counting. Half-escape times were determined directly from the first-order logarithmic plot of the escape from the retentate solution. Unless otherwise specified the half-escape times apply to a temperature of 25 ± 1°. By immersing the dialysis apparatus in a thermostated water bath half-escape times of dialysis were obtained at other temperatures.

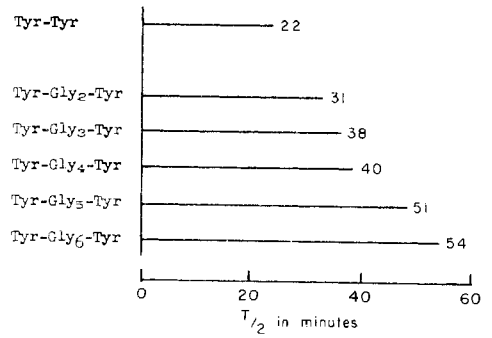

FIGURE 1: Comparative half-escape times of a series of peptides with terminal tyrosine residues separated by glycine residues.

Results and Discussion

In order to correlate thin film dialysis data unambiguously with the conformation of a polypeptide evidence must be derived that the rate of dialysis is to a first-order degree a true reflection of diffusional activity. As postulated in a previous paper (Craig, 1972), it then is based on the probability that a molecule of the solute being studied will find and enter a "pore" of the membrane. The solute must behave ideally in the membrane, *i.e.*, not interact with the membrane by adsorption or by electrostatic interaction or show self-association. By comparison of dialysis rates with those obtained with model solutes of known Stokes radius it is then possible to estimate the Stokes radius or the hydrodynamic volume of the unknown peptide to about ±3% (Craig and Pulley, 1962) provided the porosity of the membrane is adjusted to a size range giving high selectivity. Methods for detecting nonideality have been discussed in previous publications (Craig, 1967). The half-escape times given in this paper are based on straight line escape plots except where otherwise indicated.

Figure 1 is a bar plot of the comparative half-escape times of the polypeptides listed. The solvent was 0.1 M NaCl at 25°. It shows that the half-escape times in relation to the number of bridge glycine residues is not a linear function and that the tightest packing occurs when four glycines are the bridge residues in the polypeptide. Accordingly it was decided to make the remaining comparisons in this paper on the basis of hexapeptides with four identical residues serving as the bridge between the terminal residues being compared.

Figure 2 is a bar plot of the comparative half-escape times of 17 hexapeptides. In considering these data it must be realized that the selectivity increases considerably with the longer half-escape times. This has the effect of overemphasizing the differences in diffusional size particularly with the slower diffusing peptides. The solvent was 0.1 M NaCl at either pH 3.5 or 5.0. The most compact polypeptide of the series is 1, Tyr-Gly$_4$-Tyr. When the more hydrophilic residue, threonine, is substituted for a tyrosine it is interesting that a slower rate of dialysis is observed. It is logical to ascribe this to interaction with the solvent and a greater tendency for the threonine residue to extend into the solvent as compared to the tyrosine.

Peptide 3, Tyr-Ala$_4$-Tyr, dialyzes more slowly and is more rod like as would be expected (Burachik *et al.*, 1970) from the influence of the methyl groups. Here, the "allowed" (Ramachandran and Sasisekharan, 1968) rotamers are more restricted than with glycine residues. Peptide 5, Tyr-Pro$_4$-Tyr, apparently is still more rod like or extended. This is consistent with the existence of a trans arrangement of proline residues

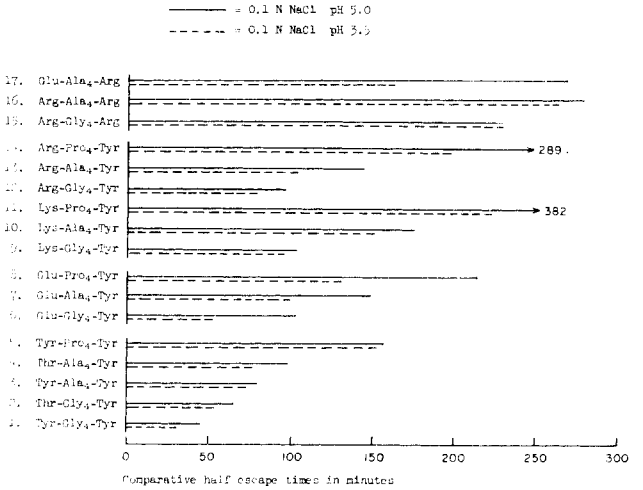

FIGURE 2: Comparative half-escape times of linear hexapeptides with four central glycine residues and various terminal residues.

in the peptide, a conformation in agreement with published data on polyprolines (Strassmair *et al.*, 1969), which are thought to favor the all-trans form in a left-handed helix in aqueous solution. Infrared studies of lyophilized peptides in KBr in agreement with published data (Blaha *et al.*, 1966) supported this view but a small fraction of the cis form could not be excluded. However, the X and Y residues could tend either to fold back on the bridge residues or if they were hydrophilic, they would be expected to extend as far as possible into the solvent and thus give a conformation with maximum extension. The data of Figure 2 appear to support this hypothesis since peptides 1–5 are observed to dialyze more rapidly than corresponding analogs 9–14 where a single charged residue replaces tyrosine or threonine, and peptides 9–14 except 11 dialyze faster than analogs 15 and 16 where the peptide contains two charged terminal residues.

It is of further interest that with peptides 6, 7, 8, and 17 a dramatic increase in dialysis rate is observed when the pH is decreased to pH 3.5 where the γ-carboxyl group of the glutamic acid becomes un-ionized. This group then becomes less hydrophilic and less extended into the solvent and the conformation becomes more compact.

Such an effect is less apparent with the other polypeptides in Figure 2 because pH 3.5 is not sufficiently low to discharge more than a fraction of the ionized C-terminal carboxyl group. For the data in Figure 2 the pH of the solvent was not controlled by a buffer. The higher pH was merely that of the distilled water and the lower pH was reached by bringing the solution to pH 3.5 by addition of the required amount of HCl.

In order to be more certain of the effect of pH a study was made in 0.1 M NaCl buffered at various pH's by 0.01 M buffer. The buffers were formate, acetate, or phosphate for the appropriate pH. The data are shown in Figure 3. Each point represents a single dialysis. With the top three peptides it is the C-terminal carboxyl that is being titrated. Tyr-Pro$_4$-Tyr appears to give a much larger shift because its diffusional size is larger and the half-escape time, therefore, falls in a more selective range since it is nearer the total exclusion point of the membrane. The curve for the bottom peptide is different because two carboxyl groups are being titrated, one of which, the γ-carboxyl of the glutamic acid residue, has a higher pK than the C-terminal carboxyl. The shift also covers a wider range as would be expected.

The methyl ester of Tyr-Gly$_4$-Tyr was prepared and studied in the same way. Its half-escape time was not influenced by

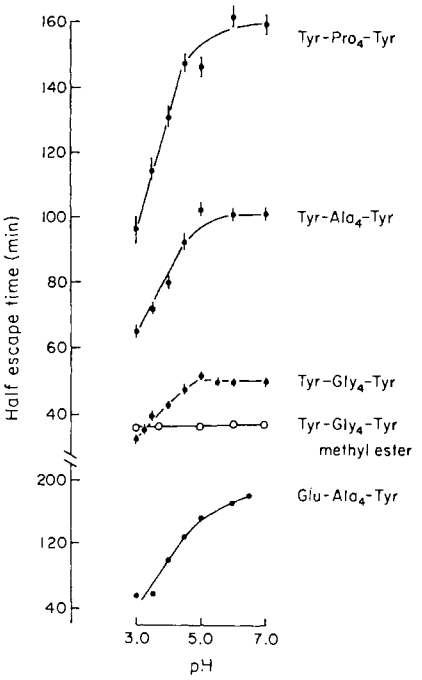

FIGURE 3: Effect of pH on the half-escape times of various hexapeptides.

change of pH in the range 3–7 and the escape rate was near that of the free peptide with the carboxyl group protonated. A pH below 3 was not studied because of the danger of losing acetyl groups from the calibrated membrane and thereby making it much more porous.

In order to accumulate more information on the effect of pH and charged groups the data in Figure 4 were obtained. In this series the basic charge of the arginine residue is removed by a nitro group. Comparison of peptide 1, Tyr-Gly-Tyr, with 2, NO_2Arg-Gly$_4$-NO_2Arg, indicates the latter to be larger as would be expected from the larger bulk of the nitroarginyl residue as compared to the tyrosyl residue. Peptide 3, NO_2Arg-Gly$_4$-Arg, indicates that removal of the NO_2 group from the C-terminal residue results in a peptide more elongated than 2 but if it is removed from the N-terminal residue also to give two like positive charges a still greater elongation results as shown by the behavior of peptide 5, Arg-Gly$_4$-Arg. A small interesting difference can be seen between peptides 3 and 4.

A similar difference is found in the X-Ala$_4$-Y series as peptides 6–9 in Figure 4 show. Each of these peptides, however, is more elongated than their counterparts in the X-Gly$_4$ series.

The comparative solution effect of pH and ionic strength is shown in Figure 5 with seven of the hexapeptides. It appears that increasing the ionic strength and thereby the polarity of the solvent tends to increase the diffusional size when X is lysine as shown by Lys-Gly$_4$-Tyr or Lys-Ala$_4$-Tyr but not when it is glutamic acid. This difference could be due in part to an electrostatic effect since the charges would not be shielded in very low ionic strength. A closer examination of the escape plots of these basic peptides revealed another explanation. In distilled water there was adsorption to the membrane and a curious acceleration of diffusion as discussed later. Glu-Ala$_4$-Arg is more extended than the first five members of this group. The data in Figure 2 show it to have about the same diffusional size as Arg-Ala$_4$-Arg. For all glutamyl peptides and especially Glu-Gly$_4$-Glu the data in Figure 5 show the effect of protonation of the γ-carboxyl group. It is also interesting that at pH 5 increasing the ionic strength

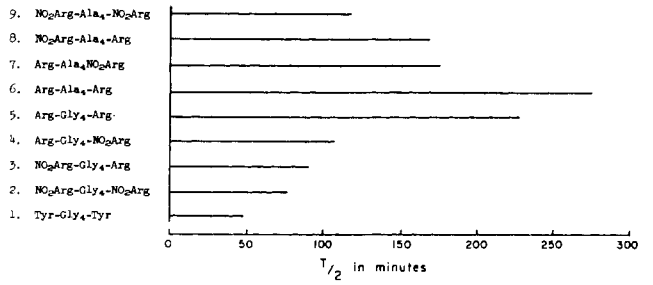

FIGURE 4: Comparative half-escape times of a series of arginine and nitroarginine peptides.

marginally slows the rate of dialysis as will be discussed later.

The effect of greatly increasing the ionic strength on Tyr-Gly$_4$-Tyr is also interesting. An ionic strength of 0.1 does not seem to have much effect as compared to distilled water but increasing the salt content to 2.0 M induces a larger diffusional size. This was found with other members of the series when X and Y do not carry two positive charges.

The effect of a change in temperature is of interest in connection with the study of linear polypeptides of conformational mobility. Studies on this problem by thin film dialysis (Craig, 1972) have shown that rigid ideal solutes follow closely the Stokes–Einstein relationship first established for free diffusion. In this the diffusion coefficient or rate of dialysis is directly proportional to the absolute temperature but inversely proportional to the change in viscosity. It has been shown (Craig, 1972) that an Arrhenius type plot is useful for these comparisons. Such a plot is shown in Figure 6 for five of the peptides. The ideal plot for a rigid solute is given for comparison. The

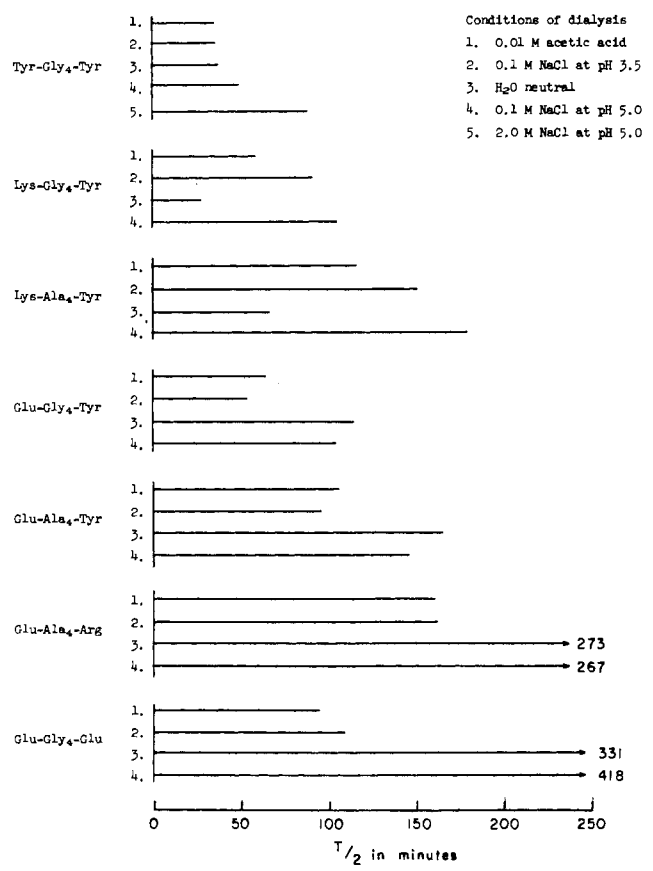

FIGURE 5: Comparative half-escape times showing the effect of different pH's and ionic strengths.

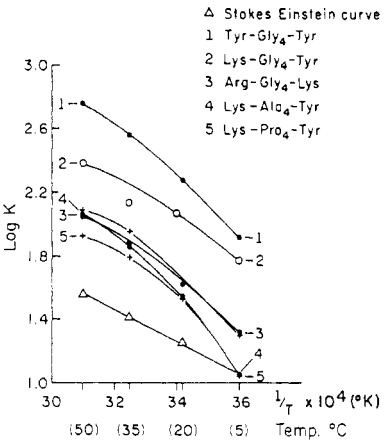

FIGURE 6: Arrhenius-type plots showing the effect of a change of temperature on half-escape times.

K used in this plot is the usual rate constant calculated from the half-escape time by the relationship $K = 0.693/\text{half-escape time}$.

Two features of these plots are of interest. One is the comparison of the curvature with the ideal plot and the other is the relative slopes of the lines or curves. If either of these differ from the ideal conformational shift or change in the degree of solvation is indicated. The relative position of the line or curve on the plot is a function of the particular porosity of the membrane. A slope steeper than the ideal indicates that the diffusional size becomes smaller as the temperature increases.

In the ideal case the slight curvature is largely due to the change in viscosity of the solvent. It is therefore of interest to construct a plot in which the K is plotted directly against the absolute temperature divided by the viscosity. This was found to give a straight line plot for each peptide but with different slopes as shown in Figure 7. The slope of this line is a function of the relationship of the diffusional size of the peptide to the porosity of the membrane. If it were an ideal solute following the Stokes–Einstein relationship a theoretical slope can be calculated for each size porosity relationship. Such a slope is that shown in Figure 7 for Tyr-Gly$_4$-Tyr. Comparison of this slope with that experimentally obtained shows the experimental one to be much steeper indicating the diffusion rate to increase linearly much faster than would be expected of an ideal solute. This could result from a decrease in Stokes radius with increasing temperature. If so it is interesting that the degree of collapse is directly proportional to the temperature.

An interesting observation to be derived from the data in Figure 2 relates to the relative importance of electrostatic interaction in the determination of conformation. It had been assumed earlier (Craig *et al.*, 1971) that charged groups of opposite sign located on each end of a linear polypeptide would tend to make it assume a more spherical conformation while charges of like sign would have the opposite effect. The behavior of peptide 17, Glu-Ala$_4$-Arg, in Figure 2 and of Lys-Gly$_4$-Glu, not listed in Figure 2, does not support this theory. Lys-Gly$_4$-Glu in 0.1 M NaCl showed a half-escape time of 316 min compared to the other peptides in Figure 2. Thus, there seems to be no tendency for these peptides to be more compact than peptides Arg-Gly$_4$-Arg and Arg-Ala$_4$-Arg, Figure 2. Electrostatic interaction, an effect treated theoretically by Flory and Schimmel (1967), appears to play a slight or at least a secondary role in determining conformation. Instead it seems to be the hydrophilicity of the charged groups and the attraction of the solvent that exerts the stronger effect on the conformation. In this comparison the charges are largely shielded by the 0.1 M NaCl.

In Figure 5 with Lys-Gly$_4$-Tyr and Lys-Ala$_4$-Tyr a low ionic strength, where the charges were not shielded, appeared to favor a more compact form. This conclusion is not warranted. Although the full explanation of the anomalous behavior is not apparent from the data presented in this paper several explanations can be offered. One is that in spite of attempts to remove all fixed anionic charges from the membrane by conjugation with glycinamide it still behaves as a weak ion exchange membrane when the solute is a positively charged peptide. Another explanation is based on a counterion effect. The basic peptides have been recovered by lyophilization from neutral solution and thus contain a counterion, usually acetate, which is considerably smaller than the peptide. In distilled water, because the small counterion has a greater tendency to diffuse into the membrane, the rate of entry of the larger basic peptide could also be accelerated. Increase in the ionic strength would be expected to depress such an effect.

If a weak ion exchange property on the membrane has the effect of accelerating entry of basic polypeptides into the membrane increasing the diffusion rate, then adsorption to the membrane should be detectable by recovery experiments. This theory is supported by the finding that toward the end of the dialysis in distilled water all the basic peptides were slow to emerge from the membrane. The fraction remaining in the membrane could be eluted more quickly with 0.1 M NaCl or with 0.01 M acetic acid than with distilled water. A degree of

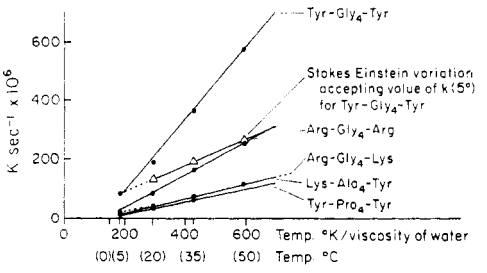

FIGURE 7: Arrhenius-type plots in which the abscissa values are divided by the viscosity of water.

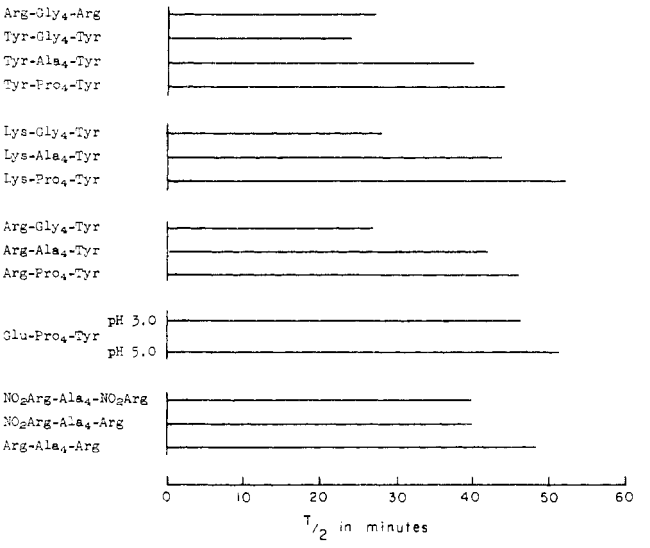

FIGURE 8: Comparative half-escape times of a series of peptides in a porous unacetylated membrane.

adsorption is thus indicated. However, if an ion exchange effect is the sole basis for the faster dialysis of the basic peptides in distilled water as compared to 0.1 M NaCl then the reverse should be found for the acidic peptides. The behavior of Glu-Gly_4-Glu, Figure 5, does not support this postulate. Instead its diffusion rate is marginally slowed by 0.1 M NaCl.

It is possible that both mechanisms are operative. In any case it appears that reliable interpretations regarding diffusional size cannot be made by this method in water at low ionic strength when the small peptide carries an excess of positive or negative charges. It is to be expected that this difficulty would become relatively less for each excess charge as the molecular weight of the peptide increases.

In attempts to establish that dialysis rates reflect mainly true diffusional size rather than unknown parameters, greater reliability can be achieved by the use of membranes with more than one selectivity. It is thus interesting to study the series of peptides used in Figure 2 in a less porous membrane, preferably one with a different surface hydrophilicity. This can be accomplished easily by the use of Visking 18, Nojax casing which can be linearly stretched (Craig and Konigsberg, 1961) to provide this minimum pore size without acetylation. As stated in the Experimental Section, no difference was found between linearly stretched and nonstretched but acetylated to the same porosity as judged by calibration. This comparison, however, was not made with all the peptides. The nonstretched membrane is more highly acetylated. Acetylation increases the hydrophobicity of the membrane and makes it less spongy when wet. Wet cellophane is in fact a rather rigid gel and acetylation increases the rigidity and strength of the gel.

It was found that linearly stretched size 18 Visking would give a half-escape time of 12 min at 25° for Tyr-Gly_4-Tyr as compared to 49 min for the acetylated membrane used in Figure 2. This rate does not provide much selectivity over that which would be provided by free diffusion (Craig and Pulley, 1962). It was, therefore, interesting to find that the discrimination between the X-Gly-$_4$-Y, X-Ala_4-Y, and X-Pro_4-Y series, shown in Figure 8, although less in this more porous, more hydrophilic membrane than for the less porous acetylated membrane, Figure 2, was greater than expected. The differences were in the same order. On the other hand, the discrimination within each series where the peptides differed by terminal residues with excess positive or negative charges was very low as was expected on the basis of porosity. Apparently a higher selectivity is achieved depending on the relative hydrophobicity of the membrane surface as compared to the solute being dialyzed. This indicates that for linear strongly basic peptides of this size dialysis rates may not always provide a true reflection of Stokes radius. For highly charged peptides an ion exclusion parameter may make interpretation difficult. The indications derived from this series must be studied further to learn more concerning the nature of the membrane solute interaction.

Such differences may also arise from differences in "pore" structure. The effective "pore" may after all not be a discrete three-dimensional irregular pocket filled with the solvent. It can be a two-dimensional tiny region on the surface of the membrane in which the cellulose chains happen to be held together more weakly than other spots on the surface by secondary forces. The relative hydrophobic–hydrophilic balance then could be important in penetration. With this hypothetical state of affairs the relative conformational stability of the peptide could also play a role since a peptide with very low conformational stability could be deformed on contact with the membrane. Highly charged small peptides whose shape in aqueous solution is largely determined by their polar groups would be expected to have a very low order of conformational stability.

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