Partition Behavior of Native Proteins in Aqueous Dextran–Poly(ethylene glycol)-Phase Systems†

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ABSTRACT: Aqueous solutions of dextran and poly(ethylene glycol) when mixed above certain concentrations give rise to aqueous–aqueous two-phase systems. Some salts partition unequally between these phases causing the phases to become charged with respect to each other. This permits one to determine a protein's isoelectric point by a number of partitions at different pH's in phases containing one of two different salts. The partition coefficients of a number of native non-hemoproteins and hemoproteins were examined in this manner to probe the factors involved in determining their partition. While exceptions to our generalizations exist, some interesting relations between partition coefficients of proteins and their relative size and structure have been found. A tendency of partition coefficients at the isoelectric point of non-hemoproteins to decrease with increasing molecular weight is in evidence. Hemoproteins have partition coefficients (at the isoelectric point) that appear independent both of molecular weight (a phenomenon not directly attributable to the heme group *per se*) and, as shown earlier, of relative electrophoretic mobility. It follows, from a formula of Brønsted, J. N. (1931), *Z. Physik. Chem., Ser. A*, 257, that interaction between the non-hemoproteins and the phases are reasonably similar and that, hence, molecular weight is the prime determinant of these proteins' partition coefficients. Hemoproteins must interact with the two-polymer phases in a manner which offsets their increasing molecular weights so as to give partition coefficients independent of them. Within the general relations described above some differences in partition coefficients at the isoelectric point between closely related proteins have been found. Hemoglobins from a number of different species and some human hemoglobin variants have different partitions (Walter, H., and Sasakawa, S. (1971), *Biochemistry 10*, 108). Hen and turkey egg-white lysozymes and β-galactosidases from two different *Escherichia coli* strains also give different partition coefficients. The basis for such differences remains unclear but may be related to the relative hydrophobicity of the proteins. Some of the information to be gleaned from protein cross-partition curves (*i.e.*, the curves obtained when plotting the partition coefficients of proteins in phases containing one of two different salts *vs.* pH) is discussed and presented.

Liquid two-phase systems with some unique properties are obtained when aqueous solutions of two different polymers (*e.g.*, dextran and poly(ethylene glycol)) are mixed at certain concentrations (Albertsson, 1971). Since each of the phases can contain more than 90% water, they can be buffered and rendered isotonic (if necessary) and have proved suitable for the partition (and separation by countercurrent distribution) of cells, cell particles, membranes, and macromolecules (Albertsson, 1970, 1971; Walter, 1969; Brunette and Till, 1971; Walter and Sasakawa, 1971).

Even though the polymers (dextran and poly(ethylene glycol)) used in the present experiments are themselves non-ionic, different inorganic salts partition differently between the phases (Johansson, 1970). As a result an electrical distribution potential between the phases is created (Albertsson, 1971). Electrophoresis of droplets of one phase in the other has demonstrated a zeta potential between them, the magnitude and sign of the charge depending markedly on the salt composition of the phase system (Seaman and Walter, 1971). It is therefore, in retrospect, not surprising that one of the major determinants in the partition of suspended materials (*i.e.*, cells and particles) is their surface charge (Walter and Selby, 1966; Walter *et al.*, 1967; Brooks *et al.*, 1971). Further the dependence of the partition coefficient of both suspended and soluble materials on the ionic composition of the phases becomes more understandable. While *sign* of charge of proteins determines the partition coefficient in a phase system of given salt composition, *magnitude* of charge is apparently outweighed by other factors (Walter and Sasakawa, 1971). The present work was undertaken to probe a few of the possible parameters that may be involved in determining the partition coefficients of proteins in these phases. While exceptions to our generalizations exist, some interesting relations between partition of proteins and their relative size and structure have been found.

It should be emphasized that partition of materials is an extremely sensitive measure of those properties that are involved in determining it. This follows from the qualitative relation derived by Brønsted (1931) many years ago (see discussion below). For example, cell partition coefficient is a far more sensitive indicator of surface-charge-associated properties than is electrophoresis in which surface charge is related linearly rather than exponentially to the measurement (H. Walter and G. V. F. Seaman, in preparation). Information on the factors involved in the partition of proteins in aqueous two-polymer phases is therefore potentially highly useful.

Methods

Proteins. Horse heart cytochrome *c*, bovine serum albumin, papain from papaya latex, α-amylase from *Bacillus subtilis*, bovine pancreatic ribonuclease A (RNase), and horse liver

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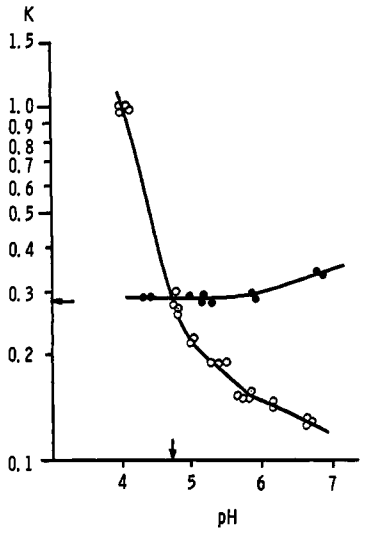

FIGURE 1: Cross-partition curves of beef serum albumin. Partition coefficients, K's, in phase system I (○, containing NaCl) and in phase system II (●, containing Na_2SO_4) are plotted as a function of pH. The arrows indicate the K and pH at the cross-point.

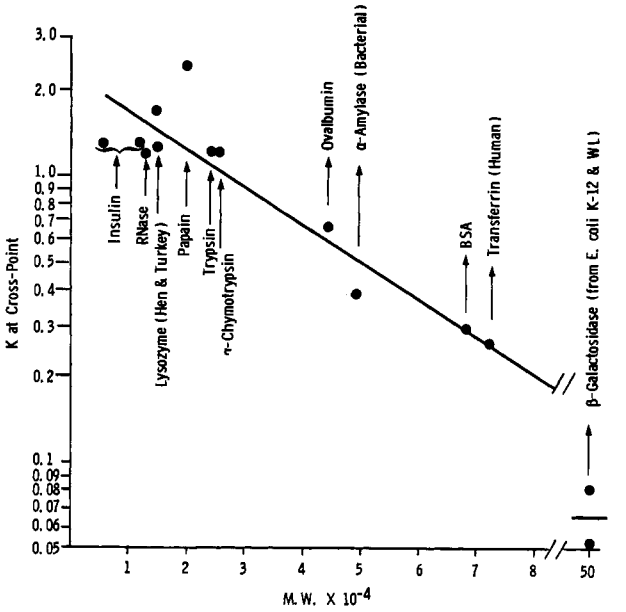

FIGURE 2: Relationship between the partition coefficients, K's, at the cross-points of 13 non-hemoproteins and their molecular weights. For details, see text.

catalase were all obtained from Sigma Chemical Co., St. Louis, Mo. Trypsin and α-chymotrypsin were purchased from Worthington Biochemical Co., Freehold, N. J. Hen egg-white lysozyme and bovine serum albumin were from Pentex, Kankakee, Ill. Hen ovalbumin was either from Schuchart, Munich, Germany, or from Sigma Chemical Co. β-Galactosidases from *Escherichia coli* K 12 and WL were kindly supplied by Dr. I. Zabin; sheep heart cytochrome *c* by Dr. A. N. Glazer; pig insulin by Dr. M. Civen; horseradish peroxidases by Dr. K.-G. Paul; human plasma transferrin by Dr. J.-O. Jeppson; and turkey egg-white lysozyme by Dr. A. Miller. All proteins were dialyzed against distilled water before use.

Chemicals. All chemicals were of analytical grade.

Preparation of Phase Systems. Aqueous dextran–poly(ethylene glycol)-phase systems, buffered and containing certain salts (see below), were used in this work. Dextran T500, batches 3202 and 5996, was obtained from Pharmacia Fine Chemicals, N. J., or Uppsala, Sweden. Poly(ethylene glycol) was obtained under the trade name Carbowax 6000 from Union Carbide, New York.

In the present study the same preparation methods were used as described in our previous papers (Albertsson *et al.*, 1970; Walter and Sasakawa, 1971). In short, stock solutions were made of dextran (20% w/w), poly(ethylene glycol) (40% w/w), 0.4 M NaCl, 0.2 M Na_2SO_4, and a series of 0.04 M buffers (glycine or sodium phosphate) spanning the pH range from 3.5 to 11.5. A mixture containing 14% (w/w) dextran and 8.8% (w/w) poly(ethylene glycol) was prepared by weighing out appropriate quantities of the stock polymer solutions. Partition of proteins was carried out as follows. The mixture (2 g) containing 14% (w/w) dextran and 8.8% (w/w) poly(ethylene glycol), 1 g of 0.4 M sodium chloride solution *or* 0.2 M sodium sulfate solution containing between 0.5 and 1% of a given protein (except where the protein quantity is otherwise indicated) and 1 g of 0.04 M buffer were weighed into small centrifuge tubes. The entire mixture was well agitated. The final phase systems prepared as described had, in addition to protein, the following compositions: 7% (w/w) dextran, 4.4% (w/w) poly(ethylene glycol), 0.1 M sodium chloride and 0.01 M glycine or phosphate buffer (system I); 7% (w/w) dextran, 4.4% (w/w) poly(ethylene glycol), 0.05 M sodium sulfate solution, and 0.01 M glycine or phosphate buffer (system II). The phase systems were centrifuged at room temperature for 10 min at 1200*g* to hasten phase settling.

Determination of Protein Partition Coefficients. The partition coefficient, K, is defined as the ratio of protein concentration (or absorbance) in top phase to protein concentration in the bottom phase. Top phase (0.5 ml) and bottom phase (0.5 ml) were carefully pipetted from the phase system in each tube, and each diluted by addition of 2.0 ml of water. The solution was mixed and the absorbance measured at 280 nm (220 nm in the case of dilute protein solutions) against a top or bottom phase blank on a Gilford spectrophotometer (Model 240) or on a Zeiss PMQ II spectrophotometer. pH was measured directly on the remaining phases.

In the case of the β-galactosidases the protein concentration was determined by assay of the enzyme activity using the method of Craven *et al.* (1965). With RNase A the protein concentration was determined both by ultraviolet absorbance (280 and 220 nm) and by enzyme activity assay (Kalinitsky *et al.*, 1959).

Results and Discussion

Relation between the Positive or Negative Charge of the Partitioned Protein and the Ionic Composition of the Phase System. The partition of charged materials in aqueous two-polymer phase systems is greatly influenced by the ionic composition of the phases (Walter *et al.*, 1968; Albertsson *et al.*, 1970; Walter and Sasakawa, 1971). Thus, negatively charged materials have, as one example, higher partition coefficients in phases containing sodium sulfate rather than sodium chloride while the reverse holds for positively charged materials (Albertsson *et al.*, 1970). When the partition coefficients, K's, of proteins obtained in phases containing either sodium chloride or sodium sulfate are plotted as a function of pH one obtains two curves that cross close to the protein's isoelectric point (Albertsson *et al.*, 1970). Such a cross-point is shown

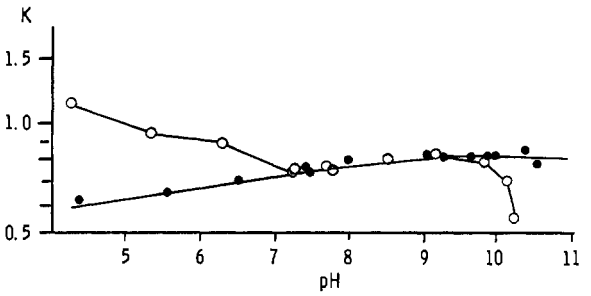

FIGURE 3: Cross-partition curves of ribonuclease A. Partition coefficients, K's, in phase system I (○, containing NaCl) and in phase system II (●, containing Na_2SO_4) are plotted as a function of pH. For details, see text.

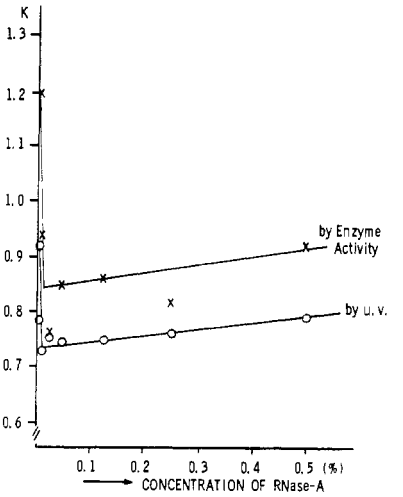

FIGURE 4: Partition coefficient, K, of ribonuclease A as a function of RNase concentration. Phase system I (containing NaCl) was used at pH 8. RNase quantity was determined by enzyme assay (×) and by uv absorbance (○).

for bovine serum albumin in Figure 1. While the ionic composition is thus shown to have a dramatic effect on the partition of a protein, its partition coefficient at the isoelectric point is virtually independent of salt composition.

Partition of Non-Hemoproteins of Different Molecular Weights. When non-hemoproteins were partitioned in a manner analogous to that described for bovine serum albumin (Figure 1), a relation between the K values at the cross-point and the proteins' molecular weights was found as shown in Figure 2. While the general correlation appears to hold for insulin (mol wt 6000), RNase A (mol wt 13,000), hen and turkey lysozymes (mol wt 14,000), trypsin (mol wt 24,000), α-chymotrypsin (mol wt 25,000), ovalbumin (mol wt 44,000), α-amylase (mol wt 49,000), bovine serum albumin (mol wt 69,000), and β-galactosidase (mol wt 500,000), papain (mol wt 21,000) is an exception.

The general phenomenon appears to be in line with Brønsted's qualitative formulation (Brønsted, 1931) that the larger the partitioned material (all other things being equal) the more one-sided the partition coefficient will be. That is, $K = \exp(\lambda M/RT)$, where K is the partition coefficient, λ is a constant characteristic of the protein-phase system interaction, M is the molecular weight, R is the gas constant, and T the absolute temperature.

While the minimum molecular weight of insulin is 6000, the protein is usually in a dimeric form (*i.e.*, mol wt 12,000) (Tristram, 1949; Mills, 1952; Velick and Ronzoni, 1948) (or even higher polymeric forms at higher pH's). We do not know the molecular weight of insulin in the aqueous phases at its isoelectric point and have therefore indicated two points (one for the monomer molecular weight and one for the dimer molecular weight) for this protein in Figure 2. Since the dimer molecular weight of insulin plotted against its K is very close to the line (Figure 2), we believe that insulin is probably in its dimeric form in these two-polymer-phase systems.

Hen egg-white lysozyme is also known to dimerize between pH 5 and 9 (Sophianopoulos and Van Holde, 1964) while turkey egg-white lysozyme does not dimerize under these conditions (A. Miller, personal communication). Whether hen lysozyme dimerizes in these aqueous phases is not known but since the K values for both hen and turkey egg-white lysozymes are in a similar range (and close to the line depicted in Figure 2) it seems that hen lysozyme dimerization does not occur in the phases and that the monomer molecular weight pertains.

Some Properties of Selected Non-Hemoproteins as Determined by the Nature of Their Cross-Partition Curves. RIBONUCLEASE A. The cross-partition curves of RNase A are given in Figure 3. The isoelectric points reported for RNase in the literature cover a considerable range. Rothen (1940) gives the isoelectric point as pH 7.8 while Barnett and Bull (1960) give a pH range from 8.2 to 9.6. It is therefore of interest that not only does RNase give a crossline rather than a cross-point (and is the only protein we have studied to do so) but that this line covers the range of isoelectric "points" obtained by other methods. Johansson (1972) has found that the divergence of the cross partition lines (*i.e.*, the lines obtained by plotting the partition coefficients of a protein in a sodium chloride phase system *vs.* pH and the partition coefficient in a sodium sulfate phase system *vs.* pH) is a function of the change of a protein's charge with pH. Since there is no appreciable change in the K value of RNase A between pH 7.2 and 9.6 in either phase system I (sodium chloride) or phase system II (sodium sulfate), we conclude that there is no net change in charge of RNase A over this pH range. Titration curves of RNase at 25° show a flat part between pH 7 and 9 (Tanford and Hauenstein, 1956; Scheraga, 1961). This finding seems to be in line with a wide isoelectric pH range.

The K values of RNase on the crossline ranged from 0.75 to 0.9 as obtained by measurement of the protein absorbance at 280 nm and 220 nm. The K value (extrapolated to 0% concentration) determined by RNase enzyme activity assay was 1.2 while it was 0.93 when determined by uv absorbance (see Figure 4). This difference in K values may be a consequence of the enzyme's impurity.

As shown in Figure 4 there appears to be a dependence of K values on RNase concentration. In the case of hemoglobins we have previously shown (Walter and Sasakawa, 1971) that the partition coefficients are independent of protein concentration except at higher alkaline pH. The independence of serum albumin partition coefficients with concentration has previously been reported (Albertsson, 1971). We have suggested that hemoglobins dissociate into subunits at low concentration in alkaline pH giving rise to the noted concentration dependence under these conditions. The basis for the dependence of the RNase K values on RNase concentration is not clear.

LYSOZYMES. Lysozymes obtained from hen and turkey egg whites show a species specific difference in their partition behavior. Figure 5 indicates the cross-partition curves obtained

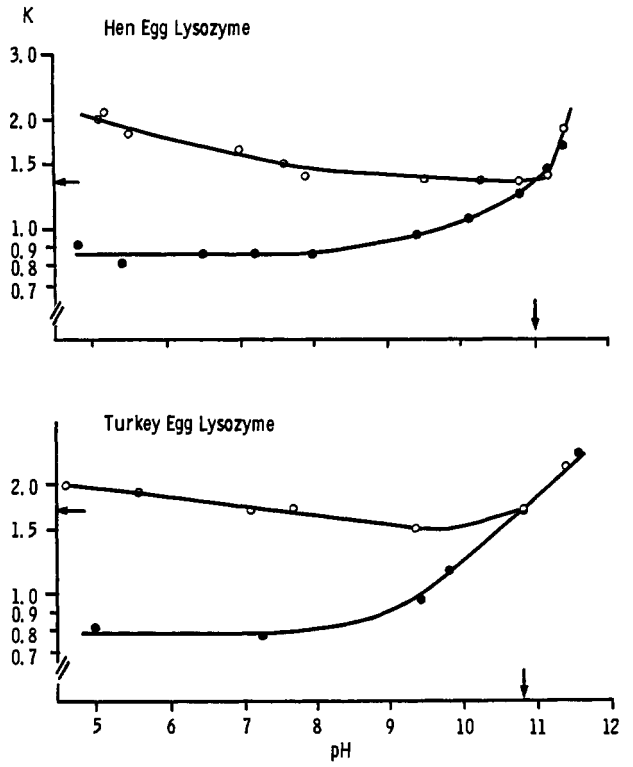

FIGURE 5: Cross-partition curves of lysozymes from hen and turkey egg white. Partition coefficients, K's, in phase system I (○, containing NaCl) and in phase system II (●, containing Na_2SO_4) are plotted as a function of pH. The arrows indicate the K and pH at the cross-point (in this case, the point at which the curves meet).

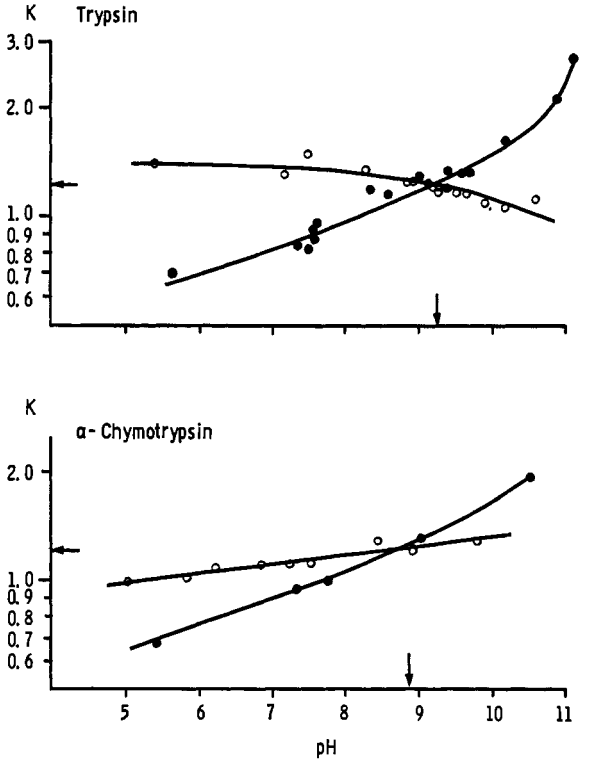

FIGURE 6: Cross-partition curves of trypsin and α-chymotrypsin. Partition coefficients, K's, in phase system I (○, containing NaCl) and in phase system II (●, containing Na_2SO_4) are plotted as a function of pH. The arrows indicate the K and pH at the cross-point. For discussion, see text.

with these two proteins. The curves, instead of crossing, just meet at the isoelectric point. Curves as depicted in Figure 5 have been obtained reproducibly and back-partition experiments (Walter and Sasakawa, 1971) in which the protein in the phase system at the highest pH is repartitioned in phases at lower pH's indicate that no irreversible alterations take place in either protein. At high alkaline pH (above 11) lysozyme undergoes denaturation (Alderton *et al.*, 1945). Hemoglobins also display rapidly increasing K's above pH 10 in both phase systems I and II (Walter and Sasakawa, 1971), a phenomenon similarly not associated with any irreversible denaturation. Turkey egg-white lysozyme gives a cross-point at pH 10.8. This differs from the isoelectric point of hen egg-white lysozyme (pH 11.0). K values are 1.3 and 1.7, respectively, for hen and turkey egg-white lysozymes at their cross-points. The difference in partition behavior between turkey and hen egg-white lysozymes is most probably a reflection of the differences in their amino acid composition, all of which are apparently at the surface of the molecules (LaRue and Speck, 1969).

PAPAIN. The only exception so far found in the relation between partition coefficient and molecular weight of nonhemoproteins is papain. The cross-point of papain is at pH 8.6 (Albertsson *et al.*, 1970) which is in good accord with its isoelectric point as reported in the literature (Smith and Kimmel, 1960). The K value at the cross-point is 2.3 which is the highest partition coefficient at the isoelectric point of any protein examined, while the molecular weight of papain is in the range of 21,000. Furthermore, the cross-partition curves (see Albertsson *et al.*, 1970) for papain are distinctly different from any obtained with other proteins. The K values of papain decrease sharply above pH 8 in both phase system I (sodium chloride) and phase system II (sodium sulfate). Other proteins show an increase in K in phase system II and a constant or slightly decreasing value of K in phase system I (except at high alkaline pH). Hence the partition behavior of papain is abnormal in phase system II. Heterogeneity as well as aggregation of papain as a function of concentration have been reported (Coates and Swan, 1970) and may be related to the high K values observed. According to the Brønsted relation (see above) aggregation (*e.g.*, dimerization) of proteins (resulting in a higher molecular weight) should (all other things being equal) make the partition of the resulting aggregate more "one-sided." Hence if the K of the monomer were above 1.0, aggregates should have higher K values; conversely, if the monomer has a K below 1.0, aggregates would have smaller K values. Subject to modification by further examination our preliminary conclusion is that papain behaves in two-polymer aqueous phases as one would expect of an aggregate or at least of a heterogeneous protein preparation (*e.g.*, mixture of monomer and dimer). Supporting such an hypothesis is the extreme variability in the amounts of sulfhydryl groups found in papain preparations which are often as low as 0.2 sulfhydryl/mole indicating the formation of mixed disulfides with glutathione and/or the dimerization of papain (A. Light, personal communication).

TRYPSIN AND α-CHYMOTRYPSIN. The K at the cross-point of both trypsin and α-chymotrypsin falls close to the line depicted in Figure 2. However, the cross-point (Figure 6) of trypsin is at pH 9.2 while its reported isoelectric point is pH 10.8 (Bier and Nord, 1950). The cross-point of α-chymotrypsin is at pH 8.9 with its isoelectric point reported as pH 8.1 (Alberty, 1967) and 8.6 (Anderson and Alberty, 1948). There is no obvious explanation why a few proteins (notably trypsin) display large differences between their cross- and iso-

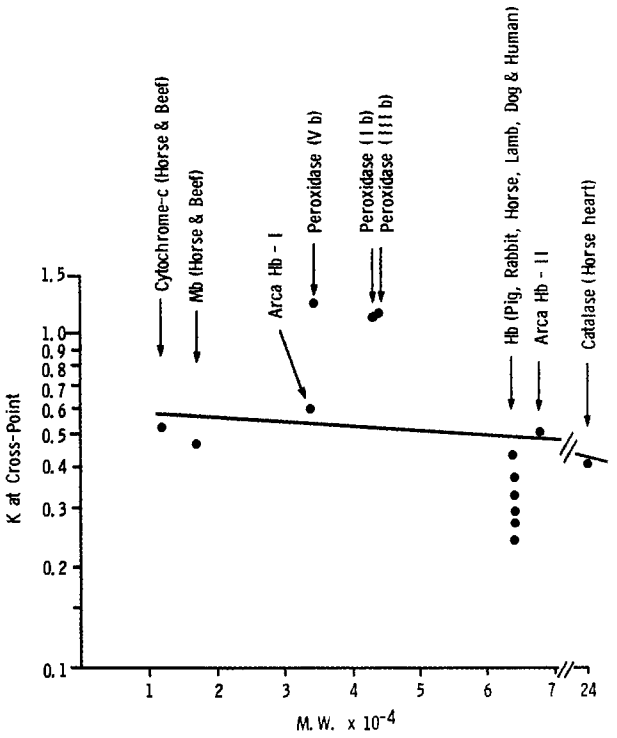

FIGURE 7: Independence of the partition coefficients, K's, at the cross-points of 16 hemoproteins and their molecular weights. The line is only drawn to emphasize the lack of a correlation between K and molecular weight. For details, see text.

electric points. However most of the proteins that show such differences have been reported to give above average differences in isoelectric points under different conditions of electrophoresis. Conceivably different molecular conformations and/or different binding of ions may be involved under various conditions of measurement which give rise not only to different isoelectric points as measured by electrophoresis but also affect the determination of the cross-point by partition.

OTHER NON-HEMOPROTEINS. Figure 2 shows a good correlation between K and molecular weight for ovalbumin, α-amylase from *B. subtilis*, bovine serum albumin, and human plasma transferrin (without iron) (see Albertsson *et al.*, 1970). β-Galactosidase from *E. coli* K 12 and WL give very low partition coefficients (*i.e.*, below 0.1) which are in line with their high molecular weights. Note that the two β-galactosidases do not have identical partitions and show strain differences, just as species-specific differences in partition behavior are apparent with mammalian hemoglobins (Walter and Sasakawa, 1971); lysozymes from hen and turkey egg-white (Figure 5) as well as with human hemoglobin variants (Walter and Sasakawa, 1971).

The above data indicate that a relation exists between the partition coefficient at the cross-point and the molecular weight of 10 out of 11 arbitrarily selected non-hemoproteins. The exception is papain. Hence for a variety of non-hemoproteins the λ factor in the Brønsted equation (see above) appears to be reasonably similar so that molecular weight predominates in determining the partition coefficient. With papain the partition coefficient obtained may be indicative of its dimerization.

Cross-partition affords an easy and accurate way to determine the isoelectric point of a protein (Albertsson *et al.*, 1970; Sasakawa and Walter, 1971). Changes in ionic strength or in salt composition have a smaller effect on the cross-point obtained by partition than in determination of the isoelectric point by electrophoresis (Sasakawa *et al.*, in preparation). The few exceptions (*i.e.*, trypsin) which have isoelectric and cross-points at different pH's may reflect subtle conformational changes that such proteins undergo in the phase system. In general the phase systems are extremely mild and no evidence for any deleterious effect on cells or denaturation of protein by the phases has been found. This is, at least in part, a consequence of the extremely low surface tension (of the order of 10^{-4} to 10^{-1} dyne/cm) between the phases (Ryden and Albertsson, 1971).

Partition of Hemoproteins of Different Molecular Weights. Hemoglobins, as previously reported (Walter and Sasakawa, 1971), show a small but significant species-specific difference in partition coefficients in two-polymer aqueous phases at the cross-point. As a class, hemoproteins from cytochrome *c* (12,000 molecular weight) to catalase (240,000 molecular weight) gave reasonably similar K values (at their respective cross-points) with the exception of some peroxidases (see Figure 7). K value of cytochrome *c* from both horse and beef heart is 0.53, 0.48 for myoglobins from horse and beef, 0.58 for hemoglobin I from *Anadara inflata* (Arca, blood clam), and 0.50 for hemoglobin II from the same source (with quite different molecular weights) (Sasakawa and Walter, 1971) and 0.41 for catalase. One can safely state that the partition coefficients at the cross-point appear to be independent of molecular weight in the case of hemoproteins. Here, considering the Brønsted equation (see above), one must conclude that, in contradistinction to the behavior of non-hemoproteins, some aspect of λ (*i.e.*, the constant characteristic of the interaction between the partitioned substance and the phases) must offset the differences in molecular weight in the case of hemoproteins.

Experiments have been undertaken to test the involvement of the heme group in determining the partition of the hemoproteins. Horse apomyoglobin was prepared by the acid acetone method of Anson and Mirsky (1930), human apohemoglobin was prepared by Teale's method (1959). The K of the apomyoglobin at its cross-point was identical with that of the horse myoglobin from which it was derived. The solubility of the human globin was extremely low and we could only get an indication of the K of the globin by partitioning the material soluble after centrifugation in phase system I (sodium chloride) or II (sodium sulfate). The K at the cross-point (measured at 220 nm) was in the range of 1.0. The partition coefficient of the globin soluble in the phases thus appears to be higher than that of the hemoglobin from which it is derived. Since the globin molecule is reported to exist as a dimer rather than as a tetramer (Rossi-Fanelli *et al.*, 1964), the molecular weight of about 30,000 (*i.e.*, the molecular weight of the dimer) with a K in this range (K = 1.0) near the cross-point would fit the curve depicted for the non-hemoproteins reasonably well (Figure 2). On the other hand, denatured proteins generally have increased partition coefficients when compared to the native protein from which they are derived (Walter *et al.*, in preparation) and the explanation for the results obtained with globin may be due to such denaturation. In any case, the lack of a correlation between the molecular weights and partition coefficients of hemoproteins can not be ascribed to the heme group alone since myoglobin and apomyoglobin have identical partition coefficients at the cross-point.

Three different isoperoxidases obtained from Paul and Stigbrand (1970) were partitioned. These three proteins have different molecular weights, isoelectric points, and carbohy-

drate contents (Paul and Stigbrand, 1970). Their K values are, however, quite similar at their respective cross-points. The latter are appreciably higher than those of the other hemoproteins as indicated in Figure 7.

Conclusion

The partition coefficients were determined of 13 non-hemoproteins and 16 hemoproteins in two polymer aqueous phases at different pH's and containing either sodium chloride or sodium sulfate. When such partition coefficients are plotted against pH, the curves obtained in sodium chloride and those obtained in sodium sulfate cross close to the isoelectric point of the respective proteins (Albertsson *et al.*, 1970; Walter and Sasakawa, 1971). At these cross-points there seems to be a relation between the K and the molecular weight of a number of non-hemoproteins (the smaller molecule having the higher K). This indicates that such proteins interact similarly with the phase system (*i.e.*, the λ value (see above) in the Brønsted equation must be similar for these proteins). With hemoproteins it appears that the K at the cross-point is independent of the hemoprotein's molecular weight, a finding not attributable to the heme group *per se*.

Within the above general correlations species-specific differences in the partition coefficients have been found not only in the case of hemoglobins (Walter and Sasakawa, 1971) but also for hen and turkey egg-white lysozymes and for the β-galactosidases from two different *E. coli* strains. Therefore, with both hemoproteins and non-hemoproteins certain subtle (or not so subtle) factors in addition to or instead of molecular weight enter into the determination of the final partition coefficients obtained. Possibly the surface hydrophobic–hydrophilic character of the proteins plays a role in their partition.

In addition to the above, partition of proteins in two-polymer aqueous phases may provide information on the dimerization or aggregation of proteins, their denaturation under certain conditions, and the change of charge on a protein as a function of pH (Johansson, 1972).[1] Experiments are being continued to further elucidate the factors that determine the partition coefficients of proteins in two-polymer aqueous phases. Since partition (see Brønsted formulation above) is a particularly sensitive measure of those factors that determine it, an understanding of the factors involved (*e.g.*, surface hydrophobicity, conformation changes, etc.) would provide their simple and sensitive assay.

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