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Behaviour of amino acids in gel permeation chromatography

Correlation with the effect of Hofmeister solutes on the conformational stability of macromolecules

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ABSTRACT

The gel chromatographic behaviour of the twenty naturally occurring amino acids was investigated. The effect of salts ranking in the salting-out side of the Hofmeister series was studied over a wide range of concentrations. The dependence of ln K_d on salt concentration was utilized to rank the amino acids in a "solvatochromic" scale which could help in predicting the propensity for regions in the polypeptide chain to be exposed to the solvent.

INTRODUCTION

The stability of biological macromolecules is a result of the sum of the interactions that take place in solution both intramolecularly and with other solutes. Since the discovery of the marked effect of neutral salts on protein solubility by Hofmeister[1] in 1888, various phenomena have been linked with this peculiar ranking of the salts not directely linked to discernible chemical or structural features of the ions[2].

The most direct consequence of, or simply relationship with, the stabilizing effect of salting-out salts in polypeptides endowed with catalytic properties is that in their presence enzymes exhibit an increased affinity for their substrates and often a higher V_{max}[3].

Another aspect, developed more recently, concerning the factors that dictate the attainment of a lower energy conformation by a polypeptide chain, is the attribution to single amino acid residues of a relative ranking of a physico-chemical property or parameter to generate a scale, such as the hydrophobicity scale, which may be of use in predicting the general behaviour of an amino acidic sequence in solution or whether

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that sequence would most likely be buried in the interior of the protein or exposed at its surface.

To quantitate hydrophobicity, many scales have been proposed (for a review, see ref. 4). Some of these scales are empirical calculations of the partitioning between the solvent-accessible surface and the buried interior in proteins of known structure. Some other scales are based on solution measurements, generally of distribution coefficients between an aqueous and an organic phase.

This paper deals with the chromatographic behaviour of the naturally occurring amino acids on gel permeation media in the presence of increasing salt concentrations. The results are interpreted in the light of a general framework linking the solvation properties of the single units of a polypeptide chain with the osmolyte composition of the solution and the attainment of an organized folded conformation of the macromolecule. A partial report on this subject was presented elsewhere as a short communication[5].

EXPERIMENTAL

Analytical reagent grade inorganic salts were used without further purification. Amino acids and ovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO, U.S.A.). Sephadex G-25 (Superfine, batch No. 6277) was purchased from Pharmacia (Uppsala, Sweden) and Bio-Gel P-2 from Bio-Rad Labs. (Richmond, CA, U.S.A.).

Water-jacketed columns (38 cm × 1 cm I.D.) were prepared from pre-equilibrated, degassed swollen suspensions of gel and the top of the column was fitted with an adjustable piston. Temperature was controlled to within $\pm 0.01°C$ with a Haake F-3C circulating water thermocryostat. Column parameters were determined with BSA and ammonium sulphate. This salt was preferred to tritiated water to measure the inner volume of the column, because with the latter an overestimate of the volume occurs owing to tritium exchange with the exchangable hydrogens in the matrix[6]. The sample (200 μl) was applied with a standardized procedure using a Pharmacia four-way valve fitted with a 200-μl loop. Each sample was chromatographed in the presence of 10 μl of 10 mg/ml BSA solution.

Elution profiles were determined by continuous monitoring at 206 nm using an LKB 2089 Uvicord III connected to a LKB 2210 potentiometric recorder. Ammonium sulphate was determined nephelometrically as its barium salt at 500 nm. Special care was taken to measure accurately the elution parameters of the column. The distance from the start line of the recording to the peak maximum was utilized, with the appropriate corrections for tubing volume to calculate the elution volume.

RESULTS

The elution positions of the twenty naturally occurring amino acids were bdetermined by aqueous column chromatography on both Bio-Gel P-2 and Sephadex G-25. The chromatographic behaviour of these small molecules, as related to the exclusion limit of the gel pores, is largely independent of classical sieving effects which are the basis of gel permeation chromatography. If a salt ranking high in the Hofmeister series (salting-out) is present in the elution buffer, the elution volume is

generally affected, to a varying extent. Moreover, this effect displays a linear correlation with salt concentration over an extremely wide range, usually limited by the salt solubility.

Relative equilibrium constants for the amino acid–gel interaction could also be calculated from the elution volumes, as it was demonstrated that these values were independent of flow-rate, thus confirming that local diffusional equilibrium was attained. Bio-Gel P-2 and Sephadex G-25 were used in these studies to avoid possible gel permeation effects (steric exclusion of the amino acids from any part of the gel).

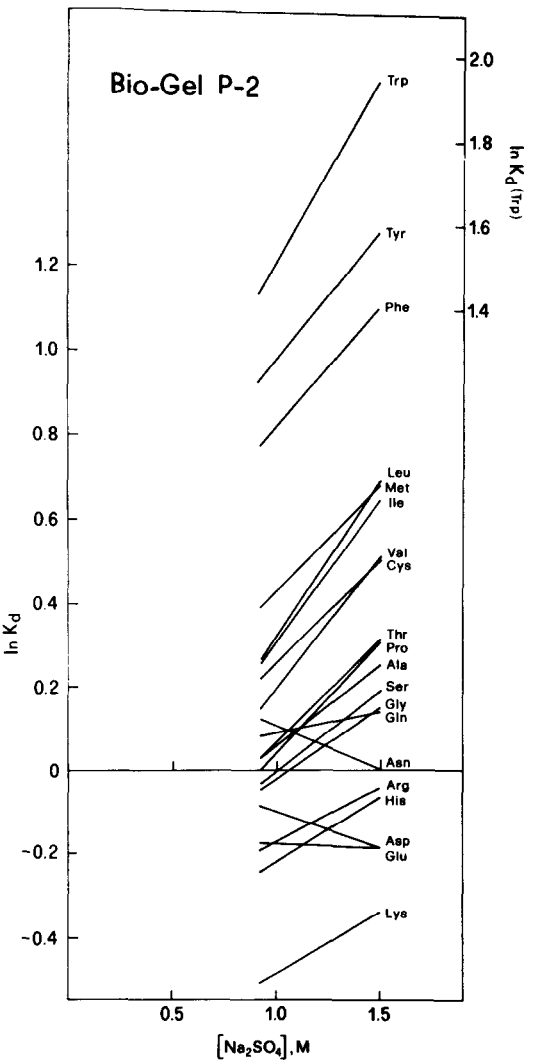

Fig. 1. Salt dependence of the distribution coefficient for the twenty naturally occurring amino acids. Amino acids were chromatographed under the conditions indicated under Experimental using Bio-Gel P-2. Temperature, 20°C.

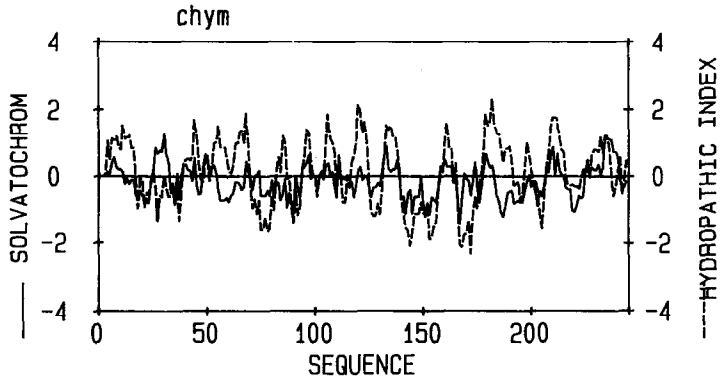

Fig. 2. Hydrophobicity plot for the chymotrypsin sequence, utilizing the Kyte and Doolitle[7] hydropathy scale and the "solvatochromic" scale deduced from the experiments shown in Fig. 1.

Fig. 1 shows the salt dependence of the distribution coefficient for the twenty amino acids when chromatographed on a Bio-Gel P-2 column using sodium sulphate as the Hofmeister solute. The same pattern and ranking were observed using Sephadex G-25. The effect is also independent of the type of salt used, provided that it has a similar ranking in the Hofmeister series. This was tested using both sodium sulphate and potassium phosphate as eluents, for both Bio-Gel and Sephadex matrices.

The ranking of the ln K_d (distribution coefficient) for the amino acids was utilized to create a hydrophobicity scale with arbitrary values ranging from -4 to $+4$ as the hydropathy scale devised by Kyte and Doolittle[7] on the basis of semi-empirical

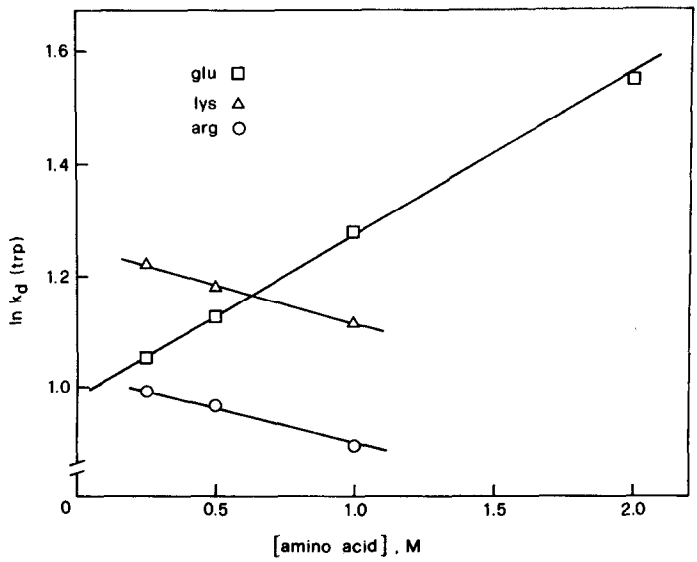

Fig. 3. Plot of ln K_d for tryptophan as a function of the concentration of the charged amino acids: (△) lysine, (○) arginine and (□) glutamic acid.

evaluations. The plot for a model protein (chymotrypsin) is shown in Fig. 2. Some similarities can be observed for certain regions in the polypeptide chain, such as at residues 135–142 or 212–220, but in general the scale displays a different overall pattern.

Among the ln K_d plots for the twenty amino acids as a function of salt concentration, as shown in Fig. 1, the behaviour of charged amino acids was noticeably different, displaying a negative dependence on salt concentration. This behaviour was not uniform among the charged amino acids and, for example, lysine displayed a marked positive correlation with salt concentration.

We therefore tested the ability of some of the charged amino acids to affect, at moderate to high concentrations, the elution position of others amino acids. The charged amino acids were thus utilized as Hofmeister solutes in the gel chromatographic experiments shown in Fig. 3. The test solute in this instance was tryptophan, for which a strong dependence on salt concentration had already been established[8,9]. Interestingly, the charged amino acids did not behave similarly. Lysine and arginine were effective, to different extents, in reducing the affinity of tryptophan for the gel. Glutamic acid, in contrast, displayed a strong positive effect on the distribution coefficient of tryptophan, behaving similarly to salts ranking high in the Hofmeister series.

CONCLUSIONS

Amino acids bind in the presence of highly salting-out salts to the gel permeation media with a rank order that is generally, bu not exclusively, dependent on the ratio of non-polar to polar groups in the compound. A linear dependence on salt concentration is present over a wide range of concentrations, often limited only by the salt solubility, as has previously been shown in the gel chromatography of some aromatic compounds[8]. With aromatic amino acids, the dependence shows a positive correlation with salt concentration. With hydrophobic or apolar amino acids, this dependence is markedly reduced and not exclusively correlated with the degree of hydrophobicity of the amino acid. A negative dependence on salt concentration is observed for a small number of neutral and for the charged amino acids.

The amplification inherent in liquid column chromatography and the enhancement of the above-mentioned effect by the presence of a Hofmeister solute is of importance for the present study. In fact, for most amino acids the expected or retarded elution is not detectable at a low ionic strength or could not be measured easily with methods such as equilibrium dialysis. Also, the adsorptive properties of the amino acids may result in a totally different ranking order when measured in the presence of moderate concentrations of a salt that is not markedly salting-out, such as sodium chloride. Moreover, the effect of different salt covering the entire range of the Hofmeister series will cause various extents of retardation with different dependences on salt concentration[9].

As we are concerned primarily with the application of this method to the development of a scale which could be advantageous in predicting exposed regions of a polypeptide sequence, we shall compare these results with those of previous studies that have dealt with the chromatographic behaviour of Hofmeister solutes.

Gel chromatography of neutral salts had been carried out under various

conditions[10,11] and in certain instances[12] utilized to calculate free energy values accounting for the stabilizing effect of a given solute on macromolecular conformation. Interestingly, the elution order of any of these salts was always found to parallel their position in the Hofmeister series, the salting-out salts being eluted earlier than expected and the chaotropes being eluted later. This effect has recently been more thoroughly investigated and correlated with the structure of the hydration shell of the ion[2].

This study has shown that the most soluble amino acids, considered to be osmolytes, may be evaluated for their effect on the solvation properties of other amino acids and, more interestingly, on the stabilization of a polypeptide chain in a solution of varying osmolyte composition. This experimental system lends itself to versatile applications such as the study of very weak interactions with a number of biologically relevant solutes, chromatographic applications or calculations of thermodynamic parameters to investigate stabilization factors in thermal denaturation processes.

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