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Protonation equilibria studies of the standard α-amino acids in $NaNO_3$ solutions in water and in mixtures of water and dioxane

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Abstract

The protonation equilibria for 20 standard α-amino acids in solutions have been studied pH-potentiometrically. The dissociation constants (pK_a) of 20 amino acids and the thermodynamic functions (ΔG°, ΔH°, ΔS°, and δ) for the successive and overall protonation processes of amino acids have been derived at different temperatures in water and in three different mixtures of water and dioxane (mole fractions of dioxane were 0.083, 0.174, and 0.33). Titrations were also carried out in water ionic strengths of (0.15, 0.20, and 0.25) $mol \cdot dm^{-3}$ $NaNO_3$, and the resulting dissociation constants are reported. A detailed thermodynamic analysis of the effects of organic solvent (dioxane), temperature and ionic strength influencing the protonation processes of amino acids is presented and discussed to determine the factors which control these processes.

Keywords: Protonation constants; Amino acids; Potentiometric studies

1. Introduction

The standard α-amino acids have special importance among the other chemical groups since they are found in all naturally occurring proteins, which play a vital role in nearly all chemical and biological processes. Despite their recognized importance, there are only a few experimental contributions on their acid–base behaviour in different environments. A search of the literature showed that the studies on the thermodynamic protonation constants of amino acids using a variety of experimental and theoretical tools have been few [1–12]. No work seems to have been done on the determination of the dissociation constants of glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, cysteine, methionine, serine, threonine, aspartate, glutamate, asparagine, glutamine, lysine, arginine, and histidine in different $NaNO_3$ solutions and in various (water + dioxane) mixtures at different temperatures.

The effect of solvents on proteins and model compounds is useful for considering how protein specific structures are stabilized in an aqueous environment. The solvation of amino acids that constitute proteins is closely connected with the stabilizing and destabilizing effects of electrolytes on protein structure; therefore, the study of dissociation and solvation processes in solutions of amino acids is important to elucidate the connection of between chemical ability and biological activity. As the polarity and the activity of water are expected to be lower in an active site cavity of an enzyme than in bulk water, the protonation processes of the studied amino acids in this investigation were examined in water containing organic dioxane solvent, from which the thermodynamic data obtained would be useful to research workers in biomedicine. Thus, in light of the above picture of the aqueous solutions of solvents, it is worthwhile to study systematically the amino acids, peptides and proteins in solvents having a different number of hydroxyl groups. These studies may shed some light

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on the mechanism about how the organic solvents affect the stability of proteins.

The stability of the native structure of proteins in aqueous solutions can be ascribed to the weak non-bonding interactions between the groups of amino acids as well as amino acids and the other components in solutions. The majority of proteins exist in aqueous mixed solvents containing many organic substances. Studies on various thermodynamic properties of amino acids and simple peptides in aqueous solutions of organic substances are of current interest due to their importance in the better understanding of the nature and mechanisms taking place in living cells. The simple heterocyclic compounds in pesticides are very important to the living organisms and the environment. The 1,4-dioxane is a type of heterocyclic compound containing an oxygen atom.

Knowledge of thermodynamic properties of amino acids is of great interest for the decoding of the mechanism of bidentate ligand dissociation and for revealing the influence of the nature of the solvent and the hydrophobicity of the alkyl radical of the acid on the energetic of processes involving amino acids. On the other hand, amino acids represent non-cyclic biologically active ligands in processes of complex formation. For the calculations of stability constants of the complex formation of amino acids with metal ions, the dissociation constants of amino acids are used. It is known that the reactions of peptides, proteins, and enzymes with metal ions are of biochemical importance but they are yet to be fully elucidated. The explanation of these phenomena in the biological systems is possible only by determining the protonation constants of the amino acids as well as their stability constants, which are the measure of their tendency to make complexes with other metal ions. The elucidation of the various phenomena in the biological systems requires the determination of the protonation constants of the amino acids and their stability constants with various metal ions in a medium similar to those of biological systems.

Generally, the dissociation constants of acids can be estimated by analysis of acid–base titrations. The methods have been critically reviewed [13–15]. Besides random errors, the systematic errors arise in instrumental measurements and the dissociation constants are obtained with limited precision and accuracy. Systematic errors are caused by limitations of: (i) the apparatus and experimental technique, and (ii) the procedure of data treatment. Both limitations introduce bias into the dissociation constants. Besides ESAB [16,17] which is one powerful programme because it permits refinement of group parameters, another programme PKPOT [18] will be used.

The purpose of this investigation is to determine the dissociation constants of the above amino acids (glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, cysteine, methionine, serine, threonine, aspartate, glutamate, asparagine, glutamine, lysine, arginine, and histidine), and study their equilibria at various temperatures in water and in different aqueous dioxane media at different ionic strengths.

2. Experimental

2.1. Materials and solutions

The standard α-amino acids were commercially available chemicals (ICN Biochemical (USA) and Aldrich, Sigma), and used without further purifications.

The B.D.H. "AnalaR" *p*-dioxane was purified by the procedure of Weissberger and Proskauer [19]. It was refluxed over pellets of KOH for about (8 to 10) h, distilled and the middle fractions of the distillate refluxed over metallic sodium for (5 to 6) h, and distilled. The middle fraction was used. Its purity was established by determining the freezing point which varied from $T = (184.75$ to $184.95)$ K (uncorrected) against the reported range of $T = (184.80$ to $185.15)$ K [20,21].

Carbonate free sodium hydroxide pellets (titrant, prepared in $0.10\ \mathrm{mol \cdot dm^{-3}}$ $NaNO_3$ solution) was standardized potentiometrically with KH-phthalate solution (Merck AG). Nitric acid, sodium hydroxide and sodium nitrate were from Merck P. A. Deionized water was used throughout the experiments.

2.2. Apparatus

The pH-potentiometric titrations were performed using a Metrohm 796 titroprocessor with a 685 dosimate, a 728 magnetic stirrer, coupled with a dosino buret model 700. The pH-titrations were carried out in an $80\ \mathrm{cm^3}$ commercial double-walled glass vessel. The ionic strength of the solutions is maintained at constant level by using the desired concentration of $NaNO_3$ solution as supporting electrolyte, and the temperature was adjusted inside the cell at the desired value by circulating thermostatted water using an oil-thermostatted set-up. During the course of titrations, a stream of oxygen-free nitrogen was passed through the reaction cell to eliminate the adverse effect of the atmospheric carbon dioxide.

2.3. Calibration of glass electrode cell

A computer programme (GLEE, glass electrode evaluation) [22] has been used for the calibration of a glass electrode by means of a strong acid-strong base titration. This programme provided an estimate of the carbonate contamination of the base, the pseudo-Nernstian standard potential and slope of the electrode and, optionally, the concentration of the base and pK_W.

2.4. Procedure for equilibrium titration

To determine the dissociation constants of protonation equilibria of the amino acids, the following solutions were prepared (total volume of $50\ \mathrm{cm^3}$) and titrated