Extraction of amino acids with di(2-ethylhexyl)phosphoric acid in the presence of dicyclohexyl-18-crown-6

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Extraction of amino acids from aqueous solutions into chloroform with di(2-ethylhexyl)phosphoric acid was studied. The extraction efficiency decreases in the sequence Trp > Phe > Leu > Arg > Lys > Ile > Ala > Gly. The addition of dicyclohexyl-18-crown-6 improves the efficiency and kinetics of extraction of most amino acids. The extraction degree depends on the concentration of the crown ether.

Key words: extraction, "host—guest" complexation; di(2-ethylhexyl)phosphoric acid, dicyclohexyl-18-crown-6; amino acids.

The development of methods of amino acid isolation is an urgent problem of biotechnology and analytical chemistry. Amino acids, as generally amines, can be extracted as "host—guest" complexes with crown ethers.[1—7] The formation of hydrogen bonds between the amino group of a substrate and the polyether cycle is the driving force of complexation. However, high polarity and hydrophilicity of a substrate often prevent the extraction of protonated amino acids. Amino acids can be extracted rather efficiently from aqueous acidic solutions into toluene[8] or benzene according to the cation-exchange mechanism in the presence of di(2-ethylhexyl)phosphoric acid (D2EHPA),[9] although the extraction capability of this reagent is insufficient for complete extraction.

In this work, we studied extraction of amino acids using both D2EHPA and a mixture of D2EHPA with dicyclohexyl-18-crown-6 (DCH18C6).

Experimental

Dicyclohexyl-18-crown-6 (reagent grade, a mixture of isomers) produced by the VNIPIM Institute (Tula, Russia), di(2-ethylhexyl)phosphoric acid (synthesized by L. K. Shpigun and T. V. Morozov, N. S. Kurnakov Institute of General and Inorganic Chemistry of the RAS), and chloroform (reagent grade) purified by a known procedure[10] were used.

Extraction was performed in glass tubes at ~20 °C. Specified amounts of amino acids were placed in tubes with ground stoppers, the required pH was established using HNO_3 and LiOH, and a solution of an organic reagent was added. The mixture was mechanically shaken during the time necessary for equilibration. The volumes of the aqueous and organic phases were 5 mL each. After the mixture was equilibrated, the phases were separated in a separatory funnel. The content of amino acids was determined in an aqueous phase by the fluorescence method using *o*-phthalic aldehyde.[11] The fluorescence intensity was measured on a Flyuorat-02 instrument (Lyumeks Scientific Production Company for Analytical Instrument-Making, St. Petersburg), and fluorescence spectra were recorded on a Hitachi spectrofluorimeter.

Results and Discussion

Extraction of amino acids by di(2-ethylhexyl)-phosphoric acid. For extraction of amino acids (Trp, Phe, Ile, Leu, Gly, Arg, Ala, Lys) with D2EHPA from aqueous solutions into the chloroform phase in the interval of pH 2—8, the time of equilibration was 30 min. The maximum amount of amino acids was extracted in the interval of pH 3—6, which corresponds to the extraction of ion associates of the protonated amino acids with the D2EHPA anion. The decrease in the extraction efficiency in the strongly acidic medium (pH < 3) is related to the protonation of D2EHPA anion, whereas that in the alkaline medium (pH > 6) is related to the deprotonation of amino acids.

At constant concentrations ($C_{AmH^+} = 1 \cdot 10^{-4}$ mol L^{-1}, where C_{AmH^+} is protonated amino acid; $C_{D2EHPA} = 5 \cdot 10^{-2}$ mol L^{-1}), the extraction of amino acids decreases in the sequence Trp > Phe > Leu > Arg > Lys > Ile > Ala > Gly, which agrees with the data[8] obtained for the extraction of Phe, Arg, Ala, and Gly from an aqueous phase into toluene, and satisfactorily correlates with the hydrophobicity of amino acids.[12] Isoleucine is extracted much worse than tryptophan, although these amino acids are close in hydrophobicity. This is likely related to steric hindrances created by a rather bulky side chain of Ile. Arginine is extracted somewhat better than the more hydrophobic lysine, probably due to the presence of guanidine group in the Arg structure, which can specifically interact with the reagent. The recovery (R) of amino acids with a $5 \cdot 10^{-2}$ M solution of D2EHPA into chloroform within the interval of amino acid concentrations of $2 \cdot 10^{-5}$—$2 \cdot 10^{-4}$ mol L^{-1} (pH 3—4) changes as follows:

Amino acid	Trp	Phe	Leu	Arg	Lys	Ile	Ala	Gly
R (%)	46	40	39	35	33	27	24	19

The presented R values are comparable with the previously published data,[8] although the reagent concentrations are much lower in our experiments.

To reveal the composition of the extracted compounds, we studied the dependence of the partition coefficients of amino acids on the concentration of D2EHPA. The slopes of the corresponding bilogarithmic dependences are somewhat lower than unity. It can be

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 5, pp. 952—955, May, 2000.
1066-5285/00/4905-0952 $25.00

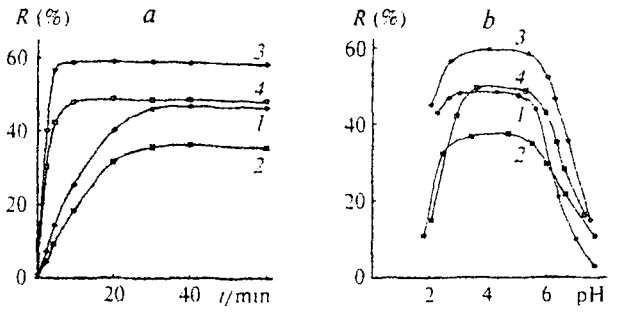

Fig. 1. Recovery (R) of amino acids ($1 \cdot 10^{-4}$ mol L^{-1}) with D2EHPA ($5 \cdot 10^{-2}$ mol L^{-1}) and DCH18C6 ($1 \cdot 10^{-1}$ mol L^{-1}) as functions of the duration (t) of phase contact (a) and pH (b): *1*, Trp—D2EHPA; *2*, Arg—D2EHPA; *3*, Trp—D2EHPA—DCH18C6; and *4*, Arg—D2EHPA—DCH18C6.

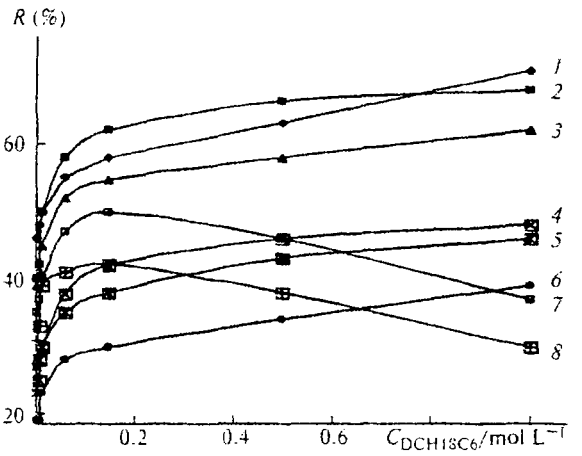

Fig. 2. Extraction of amino acids ($1 \cdot 10^{-4}$ mol L^{-1}) with D2EHPA ($5 \cdot 10^{-2}$ mol L^{-1}) as a function of the concentration (C) of DCH18C6: *1*, Trp; *2*, Phe; *3*, Leu; *4*, Ile; *5*, Ala; *6*, Gly; *7*, Arg; and *8*, Lys.

assumed that the amino acid : reagent ratio in the extracted compounds is 1 : 1. In organic solutions, D2EHPA tends to self-association. This probably explains the somewhat decreased slope of the bilogarithmic dependences.

Extraction of amino acids with di(2-ethylhexyl)-phosphoric acid in the presence of dicyclohexyl-18-crown-6. We showed for Trp and Arg that the time of equilibration in the presence of crown ether decreases to 5 min (Fig. 1, *a*), and, in addition, the presence of DCH18C6 in the extraction system favors a fast separation of the phases after extraction. The general character of the dependence of the extraction degree of amino acids on pH remains unchanged (Fig. 1, *b*).

The influence of the crown ether concentration on the extraction of amino acids into chloroform in the presence of D2EHPA is shown in Fig. 2. When the crown ether concentration increases, the recovery of most amino acids increases by 15—30%. The ratio of concentrations of D2EHPA and DCH18C6 plays a substantial role in the extraction of amino acids. For example, at $C_{DCH18C6} \leq 1 \cdot 10^{-1}$ mol L^{-1}, we observed the same order of extraction efficiency of amino acids as that for extraction with single D2EHPA. At higher crown ether concentrations, a considerable decrease in the extraction efficiency is observed for Arg and Lys, whereas the functions $R = f(C_{DCH18C6})$ for other amino acids are described by the curves reaching a plateau. This can be explained if we suppose that crown ether solvates D2EHPA: at $C_{DCH18C6} \gg C_{D2EHPA}$ all D2EHPA is solvated, which decreases its extraction capability, especially with respect to amino acids bearing two amino groups.

Analysis of the bilogarithmic dependences of the distribution coefficients of amino acids on the DCH18C6 concentration does not allow us to estimate the ratio of components in the extracted compounds, because the slopes of these functions are much lower than unity.

Thus, the presence of DCH18C6 considerably improves the extraction of amino acids by the cation-exchange reagent D2EHPA.

The authors thank B. Ya. Spivakov, L. K. Shpigun, and A. A. Formanovskii for kindly providing the reagents, and S. G. Molchan (Lyumeks NPF AP) for the use of the Fluorat-2 fluorimeter.

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Received November 3, 1999