CHARACTERIZATION OF THE HYDROPHOBIC PROPERTIES OF AMINO ACIDS ON THE BASIS OF THEIR PARTITION AND DISTRIBUTION COEFFICIENTS IN THE 1-OCTANOL–WATER SYSTEM

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The hydrophobic properties of amino acid side chains were characterized on the basis of the partition process in the 1-octanol–water system. The partition coefficients were calculated from the published data and the distribution coefficients were determined experimentally on the basis of a double partition process utilizing the fact that the amino acids pass almost completely into the aqueous phase in the partition process. When the volumes of water and 1-octanol are suitably selected, this fact permits avoidance of the difficulties associated with the determination of amino acids in 1-octanol, where their solubilities are very low. Our scale is the only complete experimental scale based on the partition process of amino acids in the 1-octanol–water system. It follows from comparison of the calculated and the experimental data with the values published for the distribution coefficients of N-acetyl amides of amino acids that the best agreement was achieved for hydrophobic amino acids, while greater differences were observed for hydrophilic amino acids. These differences, expressed as the logarithm of the distribution coefficients, correspond to an average of 0·08 for nonpolar amino acids and 0·30 for acidic and basic amino acids: expressed as relative deviations, these values correspond to 2—10% for nonpolar amino acids, and 5—30% for charged amino acids.

Hydrophobic interactions play an important role in the formation and stabilization of the native conformation of globular proteins[1]. Consequently, it became necessary to quantify the hydrophobic properties of the individual amino acid side chains. A number of experiments have been carried out in an attempt to characterize the hydrophobic properties of amino acids on the basis of study of the physico-chemical processes similar to the folding of proteins. During folding of the polypeptide chain, the amino acid side chains are transferred from aqueous medium into the relatively nonpolar interior of the protein; consequently, model processes were based on the dissolving of amino acids or analogues of side chains in various solvents[2-4], study of

the surface tension of aqueous solutions of amino acids[5], the chromatography of amino acids[6], and the partition process of amino acids and their analogues in water–organic solvent systems[7–10].

These procedures have a number of disadvantages that limit the validity of their results. There are several important limitations connected with the dissolving of amino acids in various solvents. The solubility of amino acids in various solvents is a result of competition between the solvation energy and the lattice energy of the amino acid crystals. It is known that some amino acids can exist in a number of different crystalline forms. As a result, an incorrect value can be obtained for the Gibbs energy of the transfer of the amino acid from the nonpolar solvent to water ΔG^0_{o-w} when solubility values for the various crystalline forms are employed for the calculation of ΔG^0_{o-w} on the basis of data published by various authors. In solubility measurements, the amino acids are present as saturated solutions, a very different state from the conditions pertaining during protein folding. It has been found that some amino acid side chains are even hydrated within the globular protein molecule[11]. The bonded water molecules decrease the unfavourable Gibbs energy value of transfer of the polar amino acid chains from water to the nonpolar medium inside the protein molecule[12]. However, the corresponding hydrates cannot be formed during dissolving of the anhydrous amino acid crystals in an organic solvent. The validity of results calculated from measurements of the surface tensions of amino acids[5] and chromatographic retention times[6] is limited in the study of proteins primarily from the point of view of the properties of air and the chromatographic sorbents as models of the interior of proteins. Although air is nonpolar, similar to the inside of proteins, its remaining properties are very different. Similarly, the character of the chromatographic sorbents is different from the interior of proteins. Guy[13] has stated that it is most useful to employ a partition process in a suitable solvent system as a model for protein folding.

A number of scales characterizing the hydrophobic properties of amino acids were calculated employing the fragment method from the partition coefficients of a large set of substances[14–16]. The calculations carried out so far of the partition coefficients of amino acids on the basis of their physical chemical properties and structures have not been very successful[17]. A different approach to the characterization of the hydrophobic properties of amino acids on the basis of their location in protein molecules with known tertiary structure is based on the assumption that the best models for the interior of proteins are the proteins themselves[18,19]. However, even this approach is not ideal as the internal volume of a protein is a function of its molecular weight[20] and thus the results cannot be applied unambiguously to proteins with different sizes. Similar, the use of some analogues to amino acid side chains is problematic and can sometimes lead to illogical results (e.g. the use of hydrogen in place of the glycine side chain leads to the result that the most hydrophobic side chain in the scale of Wolvenden et al.[4] is that of glycine).

The agreement between the various scales is not good and conclusions based on a given hydrophobicity scale are no longer valid for a different scale[21,22]. In addition, a great many scales do not include all the amino acids present in proteins, complicating their comparison and, together with the above-described differences between the individual scales, basically prevent comparison of conclusions obtained by different authors in studies of the relationships between the hydrophobic properties of amino acids and the protein structure.

In an attempt to avoid these difficulties, we decided to characterize the hydrophobic properties of amino acids on the basis of their partition coefficients in the 1-octanol–water system. 1-Octanol, which has a relatively nonpolar character but nonetheless contains a polar group capable of forming hydrogen bonds, would seem on the basis of crystallographic results[23,24] to be the suitable model for the interior of globular proteins, preferable to other solvents that have formerly been used for this purpose. The permittivity of 1-octanol approaches more closely the permittivity value for the protein interior. The high water content in the octanol phase permits hydrated amino acid molecules to be present in this phase. The hydration of polar and charged side chains decreases the unfavourable energy contribution of transfer of these side chains into the nonpolar medium and thus into the interior of the protein. In addition, 1-octanol is often employed as a comparative solvent in quantitative studies of the relationships between the structure and the biological activity, so that there is a considerable amount of experimental data available (some of which yield information on the amino acid side chains) so that the side chains can be compared with other substances.

This work was carried out in order to characterize the hydrophobic properties of all the amino acids occurring in proteins on the basis of their partition coefficients in the 1-octanol–water system. The partition and distribution coefficients of the side chains were calculated from the published values of the distribution coefficients of amino acids and carboxylic acids and also determined from our experiments. Because of the relatively low solubility of amino acids in 1-octanol, a double partition process was proposed for the experiments[25], based on the fact that the amino acids pass almost completely from octanol into water. Consequently, when the volumes of octanol and water are suitably selected in the second separation, the amino acid solution can be "concentrated" so that the concentration of the amino acid can be determined in the aqueous phase by some suitable analytical method. The three-component 1-octanol–water–amino acid system was employed in the partition experiments. No buffer was employed and the ionic strength was not adjusted. Consequently, the partition process reflects the properties of the amino acid alone and is not affected by interactions with other substances (e.g. the formation of ion pairs).

THEORETICAL

Assuming that partition coefficients are additive[3,27], it can be written that

$$\log P'_{\mathrm{R}} = \log P'_{\mathrm{RB}} - \log P'_{\mathrm{HB}} , \qquad (1)$$

where P' is the thermodynamic partition coefficient, R denotes the side chain, B is the matrix and H is hydrogen (the matrix is $-CH(NH_2)—COOH$ in our case). For amino acids, this relationship (*1*) expresses the dependence between the partition coefficient of the given amino acid and that of glycine.

The thermodynamic partition coefficient P' (defined as the ratio of the mole fractions) and the general (Nernst) partition coefficient P (expressed as the ratio of the molar concentrations) in the 1-octanol–water system are related by the relationship given in Eq. (*2*):

$$\log P' = \log P + \log \bar{V}^0_{\mathrm{octanol}}/\bar{V}^0_{\mathrm{water}} , \qquad (2)$$

where $\bar{V}^0$ denotes the molar volume of the solvents. Substitution of $\log P'$ from Eq. (*2*) into Eq. (*1*) yields an equation that permits the values of $\log P_{\mathrm{RB}}$ and $\log P_{\mathrm{HB}}$ to be used directly for the calculation of $\log P'_{\mathrm{R}}$, as it holds that

$$\log P'_{\mathrm{R}} = \log P_{\mathrm{RB}} - \log P_{\mathrm{HB}} . \qquad (3)$$

The values calculated from the published data are expressed as the logarithms of the thermodynamic partition coefficient of the amino acid side chain in the 1-octanol–water system. In the experimental determination, some of the amino acids can be present in various dissociated forms and consequently the results are expressed as the logarithms of the distribution coefficients of the amino acid side chains in a given system. Assuming that the distribution coefficients are equal in the first and second partition processes, $D_1 = D_2 = \langle D \rangle_{\mathrm{RB}}$, the following relationship was derived for the mean distribution coeffient $\langle D \rangle_{\mathrm{RB}}$ for the double partition process:

$$\langle D \rangle_{\mathrm{RB}} = \frac{\frac{c_2}{c_1} V_2}{V'_1 - \frac{c_2}{c_1} V'_2} , \qquad (4)$$

where c_1 denotes the amino acid concentration in the aqueous phase after the first partition process, c_2 is the concentration of the amino acid in the aqueous phase after the second partition process, V'_1 is the volume of the octanol phase from the first partition process that was then used in the second partition process and V_2 and V'_2 are the volumes of the aqueous and octanol phases in the second partition process[25].

EXPERIMENTAL

Materials

The amino acids employed were p.a. substances from the Sigma Co., St. Louis, U.S.A. Ninhydrin (Chemapol, Prague) was recrystallized prior to use. All the remaining chemicals were the p.a. substances from Lachema, Brno. Prior to use, 1-octanol was redistilled on a column with a loose packing. The measured values of the physico-chemical parameters of the rectified octanol corresponded to the tabulated values given in brackets: boiling point T_b = 195°C (195°C), density d_4^{20} = 0·826 (0·825) kg dm^{-3}, refractive index n_D^{20} = 1·428 (1·429).

Methods

A given amount of water–saturated octanol (30 to 250 ml) was added to the amino acid solution (usually 0·1 mol dm^{-3}) in octanol–saturated water (3 to 5 ml). This system was shaken in 5 min intervals with 10-fold inversion on an Erlenmeyer flask (usualy 250 ml) over a period of 1 h. The system was then centrifuged for 30 min at 1 500 g. The two phases were carefully separated, their volumes were measured and the concentration of amino acid was determined in the aqueous phase. A given amount of octanol–saturated water was added to the octanol phase (usually the same amount as the original volume of the amino acid solution, i.e. 3—5 ml.). The system was then shaken and centrifuged as described above. The volumes of both phases were again measured and the concentration of amino acid was determined in the aqueous phase. The distribution coefficients for the amino acids in the 1-octanol–water system were calculated from the values obtained for the concentration of amino acid and the phase volumes.

The analytical methods included polarography, photometry and potentiometric titrations. The distribution coefficients of most of the amino acids were determined photometrically in the visible region using the ninhydrin reaction[26]. The stock ninhydrin solution was prepared by dissolving 2·0 g of ninhydrin and 0·1 g $SnCl_2.2\,H_2O$ in 100 ml of ethanol. This stock solution was bubbled for 30 min with nitrogen and was stored in a dark ground-glass stopped bottle under nitrogen. 1·5 ml of the stock solution of ninhydrin was added to 1·5 ml of citrate buffer (pH 5·0) and 2·0 ml glycerol. 0·5 ml of this ninhydrin reagent was mixed with 0·2 ml of the amino acid solution and the mixture was heated for 10 min at 100°C on a water bath under reflux. The blue-purple solution obtained was diluted to 3 ml with a solution of 50% ethanol. The absorbance was read at 575 nm. Photometric measurements in the visible region were carried out on the Spekol 10 instrument (Carl Zeiss, Jena). The optical path length in the glass cuvettes was 1 cm.

Cysteine was determined by classical polarography using a Polariter PO 4 polarograph (Radiometer, Copenhagen) in a Kalousek cell with a reference saturated calomel electrode (SCE), to which all the potential values are related. The polarization rate was 200 V min^{-1}. The capillary had the following parameters (at a mercury reservoir height of h = 0·70 m): drop time measured at a potential of −1·50 V in the base electrolyte with composition 0·001 mol dm^{-3} $CoCl_2$, 0·1 mol dm^{-3} NH_4Cl, 0·1 mol dm^{-3} NH_3 and pH 9·3, t_1 = 2·03 s and the flow rate was m = 3·75 mg s^{-1}.

The sensitivity of classical polarography was insufficient for the determination of the distribution coefficient of cystine in the 1-octanol–water system. Consequently, differential pulse polarography was employed.These measurements were carried out on an LP 9 Polarographic Analyzer (Laboratorní přístroje Prague) with and XY Recorder (Laboratorní přístroje, Prague). The polarograms were recorded in the potential region −1·4 to −1·9 V, with a polarization rate of 300mV min^{-1}, drop time of 2 s and pulse height of 50 mV.

The distribution coefficients of phenylalamine, tyrosine and tryptophane were determined using UV spectrophotometry on the basis of measurements carried out on an SP 400 spectrophotometer (Pye Unicam) in quartz cuvettes with an optical path length of 1 cm.

Potentiometric titrations for the determination of proline were carried out using a Potassium Ion and pH Meter OP-256 (Radelkis, Budapest) with an electrode pair formed by a glass and a saturated calomel electrode. The proline samples were titrated with a 0·2 mol dm^{-3} solution of NaOH to pH 8·25.

RESULTS

The hydrophobic properties of the amino acids were characterized in this work in two ways: the partition coefficients of the amino acid side chains were calculated from the published values and the distribution coefficients were determined experimentally.

The following convention was employed to distinguish between the amino acids and their side chains: if the three-letter designation begins with a large letter, then the amino acid is referred to; if it begins with a small letter, then the amino acid side chain is being considered.

Although the partition coefficient data base is relatively large, it is not sufficiently complete to permit the use of only a single matrix **B** for the calculation of P'_R for all the amino acid side chains. Consequently, in addition to the partition coefficients of the amino acids, the partition coefficients of the amino acid hydrochlorides and carboxylic acids were also employed. Most of the data was obtained directly from measurements in the 1-octanol–water system; the remaining values were calculated from measurements in different alcohols[27]. For consistency, Eq. (*3*) was employed to compare only data obtained from a single solvent system. The published log P_{RB} values and the log P'_R values calculated using Eq. (*3*) are listed in Table I.

The results of measurements of the distribution coefficients of amino acids in the 1-octanol–water system are given in Table II. The $\langle D \rangle_{RB}$ values are the average of five determinations.

DISCUSSION

Of the wide range of approaches employed to characterize the hydrophobic properties of amino acids, the partition process in a suitable solvent system would seem most useful[13]. The 1-octanol–water system, which is used traditionally for studying the relationships between the structure and activity of substances, has been found to be useful also for characterization of the hydrophobic properties of amino acid side chains[10].

Although a number of works have been published (see Table I) characterizing the hydrophobic properties of amino acids on the basis of the partition or distribution coefficients in the 1-octanol–water system, none of them contains values for all the amino acids. This is probably because of the low solubility of these substances,

TABLE I

Partition coefficients of amino acids side chains in the 1-octanol–water system calculated from the published data

Side chain	Matrix						Average value log P'_R
	$NH_2—CH_2—COOH$		$HCl.NH_2—CH_2—COOH$		HCOOH		
	log P_{RB}	log P'^{a}_R	log P_{RB}	log P'^{a}_R	log P_{RB}	log P'^{a}_R	
gly	−3·03[e]	0[b]	−2·19[c]	0[b]	−0·54[k]	0[b]	0
	−3·00[f]						
	−3·21[g]						
	−3·11[h]						
ala	−2·74[e]	0·29			−0·31[k]	0·23	0·30
	−2·72[f]	0·28			−0·17[m]	0·37	
	2·96[g]	0·25					
	−2·74[h]	0·37					
arg	−4·44[g]	−1·33					−1·15
	4·08[h]	−0·97					
asn					−1·74[kd]	−1·20	−1·22
					−1·79[id]	−1·25	
asp					−0·70[k]	−0·16	−0·18
					0·75[i]	−0·21	
cys					0·43[r]	0·97	0·97
gln					−1·63[jkd]	−1·09	−1·09
glu	−4·51[g]	−1·30					
	−3·69[h]	−0·58			−0·59[jk]	−0·05	−0·64
his	−2·52[f]	0·48					0·11
	−4·51[g]	−1·30					
	−1·95[h]	1·16					
ile	−1·69[f]	1·31	−0·62[l]	1·57			1·44
leu	−1·55[e]	1·48	−0·69[l]	1·50			1·40
	−1·52[f]	1·48					
	−2·06[g]	1·15					
lys	−4·08[g]	−0·87					−0·40
	−3·05[h]	0·06					
met	−1·87[f]	1·13	−1·07[l]	1·12			1·12
phe	−1·35[f]	1·65			1·41[n]	1·95	1·76
	−1·52[g]	1·69					
pro	−2·54[h]	0·57					0·57
ser	−3·07[h]	0·04			−1·11[k]	−0·57	−0·27
thr	−2·94[h]	0·17			−0·64[k]	−0·10	−0·01
					−0·65[o]	−0·11	
trp	−1·06[g]	2·15			1·41[m]	1·95	2·03
	−1·11[h]	2·00					

TABLE I
(*Continued*)

Side chain	Matrix						Average value log P'_R
	$NH_2—CH_2—COOH$		$HCl.NH_2—CH_2—COOH$		HCOOH		
	log P_{RB}	log P'_R [a]	log P_{RB}	log P'_R [a]	log P_{RB}	log P'_R [a]	
tyr					0·27[p]	0·81	0·81
val	−2·10[e]	0·93	−1·17[l]	1·02			0·94
	−2·23[g]	0·98					
	−2·26[h]	0·84					

[a] log P' calculated from Eq. (*3*); [b] follows from the definition: log P'_{Gly} = log P_{HB} − log P_{HB} = 0; [c] partition coefficient value for glycine.HCl is not published, and was thus calculated from the partition coefficient for leucine.HCl according to the equation log $P_{Gly.HCl}$ = log P_{Gly} + (log $P_{Leu.HCl}$ − log P_{Leu}); [d] the partition coefficient values for asparagine and glutamine were calculated from the partition coefficients of the corresponding acids according to the equation: log P_{amide} = log P_{acid} + (π_{-CONH} − π_{-COOH}), where π_x denotes the decadic logarithm of the partition coefficient of group x (both the π_x values are taken from Table XI in ref.[27]); [e] data from ref.[28]; [f] data from ref.[7]; [g] data from ref.[8]; [h] data from ref.[9]; [i] data from ref.[29]; [j] data from ref.[30]; [k] data from ref.[31]; [l] data from ref.[32]; [m] data from ref.[33]; [n] data from ref.[34]; [o] data from ref.[35]; [p] data from ref.[36]; [r] data from ref.[37].

especially of amino acids with charged side chains, in the octanol phase, where the amino acid concentration is sometimes below the detection limit for the analytical method employed. This problem can be solved by using radioactive amino acids[9], more hydrophobic amino acid analogues[10] or a double partition process[25,38–40].

The partition coefficients of all 20 radioactively labelled amino acids have not been published, therefore it seems useful to consider the values obtained using the amino acid N-acetyl amides as the suitable standards. These values were not included in Table I but, because of their completeness, were used as reference values for both the calculated and experimental data in this work.

It can be seen from Table I that there are considerable differences among the partition coefficient values published by different authors. These differences are a result both of the different procedures employed in the determination of the partition coefficients and also of the different experimental conditions. In only a few cases were the amino acid concentrations determined in both phases; elsewhere, they were determined from the difference in the amino acid concentration in the aqueous phase prior to and after the partition process, or various analytical methods are employed for the determinations in the individual phases, decreasing the accuracy of the measurement.

TABLE II

Experimentally determined logarithms of the mean distribution coefficients of amino acids $\log \langle D \rangle_{RB}$ and the logarithms of the mean distribution coefficients of the amino acid side chains $\log \langle D \rangle_R$ in the 1-octanol–water system

Amino acid	$\log \langle D \rangle_{RB}$	$\log \langle D \rangle_R$ [a]	Amino acid	$\log \langle D \rangle_{RB}$	$\log \langle D \rangle_R$ [a]
Gly	−3·34	0	Leu	−1·84	1·50
Ala	−3·04	0·30	Lys	−4·08	−0·74
Arg	−4·25	−0·91	Met	−2·20	1·14
Asn	−3·82	−0·48	Phe	−1·43	1·91
Asp	−3·89	−0·55	Pro	−3·04	0·53[d]
Cys	−2·48	0·86	Ser	−3·53	−0·19
Css[b]	−5·08	0·80[c]	Thr	−3·25	+0·09
Gln	−3·64	−0·30	Trp	−1·33	2·01
Glu	−3·66	−0·32	Tyr	−2·49	0·85
His	−3·32	0·02	Val	−2·27	1·07
Ile	−1·81	1·53			

[a] The values of the logarithms of the mean distribution coefficients of the amino acid side chains were calculated using the relationship $\log \langle D \rangle_R = \log \langle D \rangle_{RB} - \log \langle D \rangle_{Gly}$; [b] the abbreviation Css denotes cystine; [c] the value of $\log \langle D \rangle_R$ for half-cystine was calculated using the relationship $\log \langle D \rangle_{-CH_2-S-} = 1/2 (\log \langle D \rangle_{Css}) - \log \langle D \rangle_{Gly}$; [d] the value of $\log \langle D \rangle_R$ for proline was calculated using the relationship $\log \langle D \rangle_{pro} = \log \langle D \rangle_{Pro} - \log \langle D \rangle_{Gly} + f_H$, where f_H is the fragmentary constant for one molecule of hydrogen ($f_H = 0{\cdot}23$ according to Hansch and Leo[15]).

TABLE III

Statistical analysis of the interrelationships between the calculated ($\log P_R$), experimental ($\log \langle D \rangle_R$) and reference ($\log D_R$ from ref.[10]) hydrophobic scales for amino acid side chains

Dependence	n[a]	s[b]	r[c]	a[d]	b[d]
$\log D_R - \log P_R$	20	0·33	0·94	−0·10	0·89
$\log D_R - \log \langle D \rangle_R$	21	0·16	0·98	0·00	0·85
$\log P_R - \log \langle D \rangle_R$	20	0·27	0·96	0·13	0·88
$\log P_R - \log \langle D \rangle_R$	18	0·18	0·98	0·01	1·01
$\log D_R - \log P_R$	18	0·22	0·97	0·01	0·82

[a] Number of amino acid side chains used in scale comparison, $n = 20$ corresponds to the twenty amino acids occurring in proteins, $n = 21$ corresponds to these amino acids and cystine, $n = 18$ corresponds to the basic amino acids without glutamine and asparagine; [b] standard deviation; [c] correlation coefficient; [d] coefficients in the regression equation $y = a + bx$.

The double partition process can be used to measure partition (or distribution) coefficients of substances with different affinity for the individual components of the solvent system[25,38–40]. When a suitable ratio of the solvent volumes is employed, the dissolved substance can be concentrated in the second partition process from a large solvent volume where its concentration is low into a small volume of the second solvent so that the final concentration of the dissolved substance is suitable for determination by a particular analytical method. It is advantageous that both the determinations of the dissolved substance are carried out in the same solvent (the aqueous phase for amino acids). One of the disadvantages of the double partition process is that the experimental time is also doubled and the phase volumes must be measured.

It follows from comparison of the calculated and experimental values with the values of the distribution coefficients of amino acid N-acetyl amides[10] that the best agreement is obtained for hydrophobic amino acids, while there are greater differences for hydrophilic amino acids. This difference can be explained both by the differences in the experimental conditions (pH, ionic strength, ion pair formation) and also by the fact that the concentrations of the hydrophilic amino acids in the octanol phase are two to three orders lower than for hydrophobic amino acids, so that the determination of the concentration of the hydrophilic amino acids in the octanol phase is more difficult and less accurate. The greater differences in the calculated partition coefficients for the side chains of asparagine and glutamine are a result of the partial dissociation of the carboxyl group in the side chains of aspartic and glutamic acids in the experimental studies[30,31], while this dissociation was not taken into consideration in the calculation of the partition coefficients of the side chains of asparagine and glutamine (see Table I, note *d*). It follows from Table III that, when asparagine and glutamine are omitted, the correlation between the two scales is improved. A considerable difference was observed for cysteine, where the value of the distribution coefficient of the side chain of cysteine N-acetylamide[10] is very different from both the calculated and experimental values. The value given differs from the value of the distribution coefficient of the side chain of cysteine given by these authors[10] (which is in good agreement with our values).

Nonetheless, it follows from Table III that the agreement between the scales is better than for other hydrophobic scales based on different methods of characterization of the hydrophobic properties of amino acids[21,22]. It follows from statistical analysis[41] that all the correlations in Table III are statistically significant.

Our experimental data were employed to study the relationships between the properties and location of amino acid residues in globular proteins[42] and to predict the secondary structures of membrane proteins[43]. These applications of our scale, which is the only complete experimental scale based on the partition process of amino acids in the 1-octanol–water system, demonstrate its usefulness for study

of the relationships between the hydrophobic properties of amino acid side chains and protein structure.

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