base for testing the hypothesis meaningfully. In the single study[9] where 12 loci differed in the mobility of their common alleles, 9 were faster in the 1st species than the 2nd, and 3 were slower.

More interesting are those studies comparing 3 or more species. Each study was analyzed by Friedmann's test[10], using only those loci whose most frequent allele differed in mobility in at least 1 species. At each locus, species were ranked according to allozyme mobility, and the summed ranks of each species computed. Large differences in summed ranks between species are indicative of the mobility correlations that are being looked for. The test statistic for each data set is a χ^2 with degrees of freedom equal to one less than the number of species surveyed.

A total of 5 *Drosophila* surveys and 30 vertebrate surveys proved suitable for such analysis, and of these only 2 of the vertebrate surveys gave results significant at the 5% level, indicating the existence of mobility correlations. Neither the overall vertebrate nor overall *Drosophila* χ^2-values were significant (table). However, the 2 significant surveys (table) merit further discussion. Both are unusual in that they describe intergeneric rather than intra-generic comparisons, yet only 5 of the 30 vertebrate data sets were concerned with inter-generic comparisons. The study on Icteridae (Aves)[11] involved 7 species of 6 genera, that on Cichlidae (Pisces)[12] 6 species of 4 genera.

In the absence of an a priori hypothesis that these 2 studies would yield significant results, it could be argued that in 30 surveys it is not surprising that 2 are statistically significant. In fact, the probability of obtaining, by chance, in 30 studies, 1 study with $p \leqslant 0.0026$ and 1 with $p \leqslant 0.0253$ is equal to (30) (29) $(0.9747)^{28}$ (0.0253-0.0026) (0.0026)= 0.0251. To this should be added the probabilities of obtaining even more unlikely results, values which are extremely small. Therefore we can conclude a posteriori that the probability of obtaining by chance at least 2 such significant results is less than 0.05.

Thus we have little evidence favoring the hypothesis that speciation is generally accompanied by a net alteration in enzyme change. However, we do have evidence for correlated changes in allozyme mobility between species in 2 data sets, both describing inter-generic differentiation. It may be that niche separation of species in different genera is generally greater than that of species in the same genus, and therefore any selection for an overall alteration in enzymic charge is likely to be stronger and more readily apparent. It follows that if charge per se is subject to selection, then the results from these 2 studies may be viewed as good evidence for the selectionist hypothesis. However, the majority of surveys accord with expectations of the neutral model in not showing consistent differences in allozyme mobility between related species.

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Hydrophobic properties of model compounds with peptide-like chemical environment: N-acetyl-N′-methyl-amino acid amides

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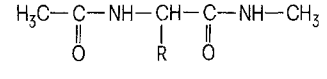
Summary. Capacity factors in reversed-phase HPLC and distribution constants in octan-1-ol/water of N-acetyl-N′-methyl-amino acid amides have been measured as a function of temperature. The HPLC capacity factors are proposed as estimates of the hydrophobicity of the amino acid side chains.

Hydrophobic effects are predominant in determining a number of different physicochemical and biological properties of peptides and proteins. The stabilization of the native conformation of proteins[2-4], the binding of biologically active peptides to enzymes and receptors[5] and the retention of small peptides in reversed-phase liquid chromatography[6] are among a few instances where the hydrophobicity of the amino acid residues is thought to play a substantial role.

Current hydrophobicity scales for the amino acid side chains are derived from various experimental data on the amino acids, e.g. solubility in alcohols[3,7], partition between immiscible solvents[8,9], chromatographic retention[10,11] and surface tension[12]. It is open to question, however, whether these scales measure the hydrophobicity of the side chain itself, since the zwitter-ionic nature of the amino acids is likely to affect hydrophobicity, for example by changing the type and extent of hydratation. Moreover, in no case was unambiguous evidence presented that a true, thermodynamically defined hydrophobic effect had been measured. Ideally, true hydrophobicity is characterized by a large positive excess of entropy change due to desolvation of apolar molecules and concomitant loss of water structure[2,13].

We have set out to measure the hydrophobic properties of N-acetyl-N′-methyl-amino acid amides with the general formula:



These compounds are un-ionized and may be considered as small peptide fragments, since the single amino acid side chain, (R), is contained between 2 amide bonds, much the same as it is in peptides.

We present here the results of 2 hydrophobicity-related measurements, viz. of retention in reversed-phase high performance liquid chromatography using octadecyl modified silica as the support, and of distribution in the classical octan-1-ol/water system. To acquire an understanding of the thermodynamics governing retention and partition, temperature functions have been determined to enable us to calculate the relevant thermodynamic parameters.

High performance liquid chromatography was carried out using customary procedures and apparatus. The 9 cm long, 3 mm bore stainless steel column was packed with 10 µm particle-size Lichrosorb RP-18 (Merck). Compounds were eluted with 0.1 M ammonium-phosphate buffer (pH 2.1) and detected at 215 nm. Measurements were made at a number of different temperatures between 20 and 60 °C. Van 't Hoff plots of log k′ vs T^{-1} (k′ is the capacity factor and T the absolute temperature) showed good linearity with $r \geqslant 0.992$, except for lysine, presumably due to its cationic side chain (fig. 1). Slopes obtained were positive with corresponding negative enthalpies of transfer to the alkyl-bonded stationary phase (see table). Intercepts contain the negative term ln Φ (Φ is the phase ratio of the column), which cannot be evaluated unambiguously[14], but is probably large enough to produce positive entropies in at least some cases.

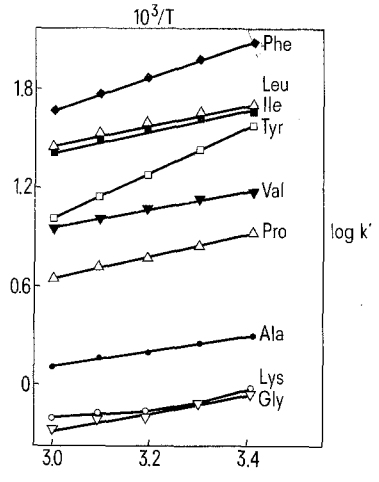


Figure 1. Van 't Hoff plots of reversed phase HPLC capacity factors (log k′) for N-acetyl-N′-methyl-amino acid amides.

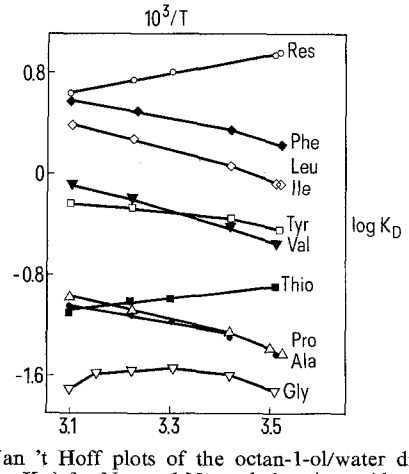


Figure 2. Van 't Hoff plots of the octan-1-ol/water distribution constants (log K_D) for N-acetyl-N′-methyl-amino acid amides and for resorcinol (Res) and thiourea (Thio).

For the partition experiments appropriate volumes of 0.1 M ammonium-phosphate buffer (pH 2.1) and octan-1-ol were vigorously stirred in a thermostated glass vessel. The UV-absorbance of the water layer was continuously monitored at 215 nm, using for the separation of the phases the filter-probe method described by Cantwell and Mohammed[15] and by Kinkel et al.[16]. Distribution constants were independent of the phase volume ratio of octan-1-ol to water and of the concentration of the amino acid derivative, which was less than 2.10^{-3} M in the water layer. Van 't Hoff plots for the partition of the amino acid derivatives exhibited good linearity ($r \geqslant 0.992$) when the low temperature points at about 12 °C were excluded (see fig. 2). Enthalpies and entropies in the table have therefore been calculated for the temperature interval of 19–50 °C. Close inspection however shows a consistent trend toward curved (convex) van 't Hoff plots, which is most strongly marked for glycine. This unexpected behavior of acetyl-methylglycinamide was confirmed with a shake-flask method and off-line detection. To check the method for any systematic deviations, distribution constants for resorcinol and thiourea were determined. The results compared favorably with existing data from other authors[17,18] using conventional shake-flask procedures. Van 't Hoff plots show excellent linearity with $r \geqslant 0.999$ and negative enthalpy and entropy changes (see fig. 2 and table).

The temperature dependence of our chromatography data is in good agreement with what is commonly found[19]. Solute retention in reversed-phase chromatography has been postulated to be a true hydrophobic process in spite of the negative enthalpies invariably found[19,20]. We are confident therefore that our chromatography-based scale reflects true hydrophobicity of the amino acid side chain in the best possible way. This is supported by the good correlation (r = 0.995) found with calculated hydrophobicities of the amino acids using Rekker's fragmental constants[21]. Since the latter are ultimately based on experimental partition data of a large number of different compounds, specific nonhydrophobic partition effects are likely to cancel out in Rekker's hydrophobic fragmental constants. On the other hand we feel that our octan-1-ol/water distribution constants do not measure a pure hydrophobic effect. The octan-1-ol/water partition system has been subject to criticism mainly for the large and temperature-dependent solubility of water in octan-1-ol and for the possible existence of specific exothermic solute-solvent interactions in the

Slopes, intercepts and capacity factors (k′) or distribution constants (K_D) at 25 °C calculated from van 't Hoff plots of reversed-phase HPLC and of octan-1-ol/water distribution data, respectively

	Reversed-phase HPLC			Octan-1-ol/water		
	ΔH°	ΔS°+R lnϕ	log k′	ΔH°	ΔS°	log K_D
Phe	−19.7	−27.1	2.304	13.1	51.7	0.401
Leu	−11.6	− 6.87	1.670	18.9	66.1	0.137
Ile	−11.9	− 8.52	1.645	20.1	69.9	0.135
Tyr	−26.3	−59.5	1.496	7.41	18.7	−0.321
Val	−10.6	−13.3	1.154	19.9	60.1	−0.348
Pro	−13.2	−27.4	0.885	16.3	31.7	−1.190
Ala	− 8.59	−23.6	0.272	12.7	19.3	−1.213
Gly	−10.1	−35.5	−0.088	-	-	−1.560*
Lys	-	-	−0.073*	n.d.	n.d.	n.d.
Res	n.d.	n.d.	n.d.	−14.1	−31.4	0.827
Thio	n.d.	n.d.	n.d.	− 8.75	−47.8	−0.963

Slopes and intercepts have been multiplied by 2.303 R (R is the gas constant) to give free enthalpies (kJ · mole^{-1}), entropies (J · K^{-1} · mole^{-1}) or values of the term ΔS°+R ln ϕ (ϕ is the phase ratio of the column). Res = resorcinol, Thio = thiourea. * Interpolated between nearest experimental points.

octan-1-ol phase[16,22]. Although the positive enthalpies and entropies we find for the octan-1-ol/water partition point to a hydrophobic partition mechanism, the non-linearity of the van 't Hoff plots and the poor correlation ($r = 0.972$) with values calculated from fragmental constants raise considerable doubts as to the nature of the effects actually measured.

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Paradoxical effect of 1-β-D-arabinofuranosyl cytosine triphosphate on bleomycin-induced unscheduled DNA synthesis in permeable sarcoma cells[1]

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Summary. 1-β-D-Arabinofuranosyl cytosine-5'-triphosphate (araCTP), an inhibitor of DNA synthesis, paradoxically enhanced unscheduled DNA synthesis (USD) induced by bleomycin in permeable mouse sarcoma cells. A greater enhancing effect of araCTP on bleomycin-induced USD was observed with lower concentrations of dCTP in the assay mixture. USD measured without bleomycin in nuclei isolated from mouse sarcoma cells was not enhanced, but inhibited by araCTP.

1-β-D-Arabinofuranosyl cytosine (araC) is used as an antitumor agent owing to its inhibitory effect on cellular DNA synthesis. The inhibitory effect has been studied in vitro using its active form, araCTP[2]. AraCTP is known to inhibit preferentially replicative DNA synthesis, but USD is also inhibited by araCTP at high concentrations[3]. Bleomycin, which is also an antitumor agent[4], is known to induce USD[5,6]. The present communication concerns the enhancing effect of araCTP on bleomycin-induced USD in permeable cells.

Materials and methods. Mouse ascites sarcoma (SR-C3H/He) cells were permeabilized by treatment with buffer B (0.25 M sucrose, 10 mM Tris-Cl, 4 mM $MgCl_2$, 1 mM EDTA and 6 mM 2-mercaptoethanol, pH 8.0) supplemented with Triton X-100 at 0.0175% (Triton-buffer B)[7]. Nuclei were prepared from SR-C3H/He cells in stationary phase and from livers of adult, male Donryu rats[8]. Permeable cells or nuclei were distributed in assay tubes at 2×10^6 cells or 4×10^6 nuclei per tube. The suspension volume was adjusted to 0.38 ml by adding Triton-buffer B for permeable cells or by adding buffer B for nuclei. For replicative DNA synthesis, 0.2 ml of substrate mixture (0.1 M Tris-Cl, 7 mM $MgCl_2$, 0.24 M NaCl, 7.5 mM ATP, 0.15 mM dATP, 7.5 μM dCTP, 0.15 mM dGTP and 7.5 μM [^{3}H]dTTP, 0.5 Ci/mmole, pH 8.0) was added to the suspension[7]. For USD, ATP was omitted from the above replicase substrate mixture. Bleomycin A_2 and araCTP, both dissolved in distilled water, were added either singly or together to the suspension in a volume of 0.02 ml[9]. The reaction mixture (final volume: 0.6 ml) was incubated at 37 °C for 10 min for replicative DNA synthesis and for 60 min for USD. The radioactivity incorporated into acid-insoluble material was measured by a disc method[10].

Results and discussion. DNA synthesis measured in permeable cells in the presence of ATP, 4 deoxynucleoside triphosphates (dNTPs), Mg^{2+} and a proper ionic environment was S-phase specific, and the DNA synthesis was due largely to the elongation of strands initiated in vivo[7,10]. All available evidence indicated that the DNA synthesis was replicative[7,10]. Replicative DNA synthesis was highly dependent on ATP and was reduced to about 5% by omission of ATP. Bleomycin induced USD, concomitantly inhibiting replicative DNA synthesis[9]. DNA synthesis measured in permeable cells in the presence of bleomycin with an ATP-free assay mixture was unscheduled[9]. USD was also induced without bleomycin in nuclei isolated from SR-C3H/He cells in stationary phase or from rat livers[8,11]. The induction was attributed to DNA damage which occurred in the process of nuclear preparation and to repair of the damaged DNA[8,11]. The unscheduled nature of the DNA synthesis was confirmed by autoradiography. USD in the present systems was clearly distinguished from replicative DNA synthesis by aphidicolin[12]. Aphidicolin inhibited replicative DNA synthesis selectively and almost completely. USD in bleomycin-treated permeable cells and in nuclei isolated from SR-C3H/He cells or rat livers was completely resistant to aphidicolin. Replicative DNA synthesis in permeable cells was highly sensitive to araCTP, whereas USD