# Unusually strong lipophilicity of 'fat' or 'super' amino-acids, including a new reference value for glycine

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*Summary.* Lipophilic, steric, electronic, and enzyme resistance characteristics of carboranylalanine, adamantylalanine, neopentylglycine and tert-butylglycine are described. The first 2 amino-acids display lipophilicities 2 orders of magnitude higher than tryptophan.

The lipophilicities (hydrophobic parameters, $\pi$)[2] of the amino-acid side chains have recently been evaluated by considering the side chains as C($\alpha$)-substituents of glycine and using partition and chromatographic data[3]. The $\pi$-values constitute a very useful measure of relative substituent hydrophobicities and enable one to separate lipophilic from electronic or steric effects[2]. They are generally calculated from the partition coefficients, P, of the parent compound and of its substituted derivative in the model solvent system n-octanol/water. In the case of amino-acids, the equation which defines $\pi$ is:

$$\pi \text{ (side chain)} = \log P \text{ (amino-acid)} - \log P \text{ (glycine)}.$$

When using this equation, a good reference value for log P (glycine) is of crucial importance. This parameter was redetermined very carefully with the help of a ninhydrin assay and was found to be 0.21 log P units lower than that reported in the literature[4].

In a quest for artificial amino-acids that will impart new, well-defined features to synthetic peptides, we have recently prepared L-o-carboranylalanine (Car)[5], L-adamantylalanine (Ada)[6], L-$\beta$-methylvaline (t-butylglycine, Bug)[7,8] and L-$\gamma$-methylleucine (neopentylglycine, Neo)[9] by chemical synthesis (figure). These 4 residues have been called 'fat' amino-acids as they exhibit enhanced lipophilicity (fatty) and altered steric requirements (bulky)[10].

Because of their considerable interest as probes for structure-activity relationships among peptides such as $\alpha$-chymotrypsin inhibitors[11], enkephalins[8,9,12,13], angiotensin II[14], bradykinin[14], substance P[14], parathyroid hormone[15], oxytocin[16] and vasopressin[16], we have measured the hydrophobic parameters, $\pi$, of Car, Ada, Bug, and Neo in order to assess the influence of their lipophilic features on enzyme and receptor binding.

The new amino-acids have unusually strong lipophilic properties as revealed by the direct measurement of their partition coefficient P in n-octanol/water or by comparison of chromatographic data.

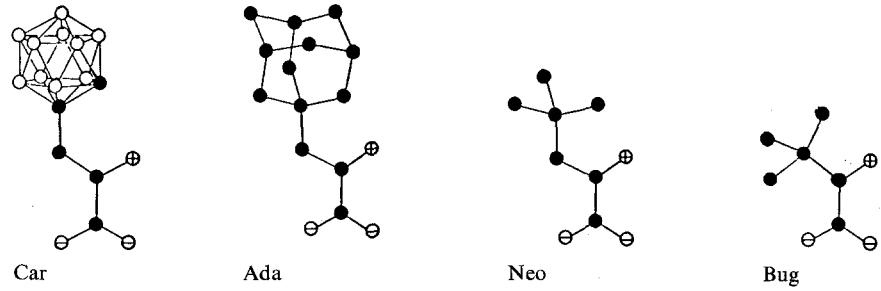
In order to determine P, the compound (about 1 mg) is dissolved in 1 of the 2 phases of the n-octanol/water system at low concentration ($10^{-3}$ moles/l). After addition of a measured volume of the other phase, the solute is partitioned by shaking for 2 min. Centrifugation (2000 rpm, 20 min) causes a complete separation of the 2 phases. The concentration of the amino-acid is directly measured in the aqueous phase only, while its content in the organic phase is calculated as the difference to the total amount of solute. The quantitative determination of the amino-acid is carried out by incubating aliquots of the aqueous phase with a standard ninhydrin solution (SIGMA) under defined conditions (15 min in a boiling water bath, then dilution with 5 vol. of a 1:1, vol./vol. ethanol/water mixture) followed by measuring the absorbance at 570 nm (room temperature). The reference is a calibration curve obtained under the same conditions, without partitioning. Each P-value is measured for 3 dilutions of 4 different samples. Since in the case of Car, Ada, Bug, and Neo no concentration dependence was observed, P was taken as the mean value of all measurements and the standard deviation calculated.

The results, together with those of other authors, are shown in the table. It is apparent that Car and Ada are the most hydrophobic amino-acids known to date. Their side chains are 112 and 31 times, respectively, more hydrophobic than the side chain of tryptophan. Bug is intermediate between methionine and isoleucine while Neo is even more lipophilic than phenylalanine. The enormous lipophilicities of Car and Ada will tend to overcompensate steric hindrance

Side-chain hydrophobic parameters $\pi$ and log P values of representative amino-acids for the octanol/water system

| Amino-acid | log P | $\pi$ |
|---|---|---|
| Glutamic acid anion | −4.51[a] | −1.30 |
| Histidine cation | −4.51[a] | −1.30 |
| Arginine cation | −4.44[a] | −1.23 |
| Lysine cation | −4.08[a] | −0.87 |
| Glycine | −3.21±0.01 | 0.00 |
| Alanine | −2.96±0.01 | +0.25 |
| Valine | −2.23±0.02 | +0.98 |
| Leucine | −2.06±0.05 | +1.15 |
| Methionine | −1.87[b] | +1.34 |
| t-Butylglycine (Bug) | −1.77±0.11 | +1.44 |
| Isoleucine | −1.69[b] | +1.52 |
| Phenylalanine | −1.52±0.03 | +1.69 |
| Neopentylglycine (Neo) | −1.42±0.04 | +1.79 |
| Tryptophan | −1.06±0.04 | +2.15 |
| Adamantylalanine (Ada) | +0.43±0.04 | +3.64 |
| Carboranylalanine (Car) | +0.99±0.02 | +4.20 |

[a] Derived from the corresponding, chromatographically determined $\pi$-value (Pliška and Fauchère[3]) using the log P-value of glycine contained in this table. All other log P-values were obtained from partition experiments as described, and the $\pi$-values calculated from them. [b] Literature value of Klein, Moore and Smith[4].




A diagrammatic representation of o-carboranylalanine (Car), adamantylalanine (Ada), neopentylglycine (Neo, $\gamma$-methylleucine), and t-butylglycine (Bug, $\beta$-methylvaline). The hydrogen atoms are omitted; ● represents carbon, ○ boron, ⊕ nitrogen, and ⊖ oxygen.

of binding to hydrophobic recognition sites of enzymes and receptors.

Finally, it is remarkable that Car, Ada, Bug, and Neo can very efficiently stabilize enekphalin peptides against degradation by various proteolytic enzymes, if introduced in position 5[8,9].

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## Distribution of cytoskeletal elements in cultured skin fibroblasts of patients with Duchenne's Muscular Dystrophy[1]

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*Summary.* Cultured fibroblasts from patients suffering from Duchenne's Muscular Dystrophy were examined by indirect immunofluorescent techniques using antibodies against actin, myosin, tubulin, and intermediate-sized filaments. The cells display normal patterns of microfilamentous bundles (stress fibres), microtubules, and intermediate-sized filaments suggesting a normal organization of these cytoskeletal structures.

Although the primary defect of Duchenne's Muscular Dystrophy (DMD) is still unknown, evidence has been presented suggesting membrane defects in tissues of neither myogenic nor neurogenic origin[3-5]. In view of the well documented interrelationships of membrane dynamics and cytoskeletal elements[6-11], Shay and Fuseler[12] studied fibroblasts from explants from cardiac and skeletal muscle of dystrophic inbred chicken and found a reduction of the immunofluorescent staining of interphase microtubules compared with those in cells of normal chickens.

We have extended the studies on cytoskeletal elements in DMD and compared the distribution of actin, myosin, tubulin and intermediate-sized filaments in cultured fibroblasts from patients with DMD and from normal persons.

The fibroblasts were obtained from biopsies of 4 patients with clinically confirmed DMD and of 4 normal, sex- and age-matched children (from the Department of Pediatric Surgery of the University of Berne). Cells were grown in 250-ml Falcon flasks in Eagle's minimum essential medium supplemented with 10% fetal calf serum and nonessential amino acids. The cultures were incubated at 37 °C in 95% air and 5% $CO_2$, and medium was changed twice weekly.

The fibroblasts, used at the same number of generations, were grown on glass cover-slips and examined as nonconfluent cultures. For the visualization of actin and myosin, cells were fixed with ethanol (30 sec, room temperature) and then briefly rinsed in PBS. For microtubular structures and intermediate-sized filaments, fixation was in methanol (5 min, -20 °C) followed by a short immersion in acetone (−20 °C). Then the preparations were air dried.

We used: 1. human anti-actin antibodies[13,14] purified by affinity chromatography, at a concentration of 80 µg/ml; 2. rabbit antiserum containing antibodies against human uterine smooth muscle myosin; the titer of the serum, measured in rat intestinal smooth muscle, was 1/1280[15]; 3. rabbit antiserum containing antibodies against pig brain tubulin[11]; 4. rabbit serum containing auto-antibodies against intermediate-sized filaments of fibroblasts[16]. All 3 sera were used at dilutions 1:10 in phosphate buffered saline (PBS; $KH_2PO_4$ 14 mM, $Na_2HPO_4$ 53 mM and NaCl 154 mM, pH 7.2).

Incubation with anti-actin and antimyosin antisera was at room temperature for 15 min; incubation with antitubulin and anti-intermediate-sized-filaments sera was at 37 °C for 45 or 60 min. After washing, the cells were further incubated for actin, with fluorescein conjugated Ig fraction of a goat serum against human IgG (Miles Seravac, Lausanne, Switzerland, dilution 1:5); for myosin, tubulin and intermediate-sized filaments, with fluorescein conjugated Ig fraction of a goat serum against rabbit IgG (Behring-Werke, Marburg Lahn, West Germany, dilution 1:20). Incubation was 15 min for actin and myosin (room temperature) and 30 or 45 min for tubulin and intermediate-sized filaments (37 °C). Cells were observed with a Zeiss UV microscope equipped with epiillumination and specific filters for fluorescein. Photographs were taken with a plan Apochromate 40× objective, using Ilford HP5 black and white film.

Upon microscopic examination, we did not detect any differences in the staining pattern of cytoskeletal proteins between fibroblasts from patients with DMD and normal subjects (figure). Decoration with antibodies to actin and myosin revealed thick stress fibres extending over long distances of the cell body (figure, a, b, e, f). As depicted in the figure (b and f), stress fibres appeared stippled after staining with antimyosin antibodies, which is characteristic