quencies of hantaviruses Hantaan strains 76118 (GenBank numbers M14627 and Y00386), Lee (D00377), and Jojo (D00376); Seoul strains B-1 (X53861), SR-11 (M34882), and R22 (S80353); Fushan strain GB18-20 (M09270) and Satoko (X61034); and Prospect Hill strain PHV-1 (X5129) were aligned with the GAP, PILEUP, and LINEUP programs of the GCG software package (Genetics Computer Group, Madison, WI) run on a VAX computer. Predicted conserved positions for the synthesis of nested RT-PCR oligonucleotide primers for HTN-SE viruses or FUU-PU viruses were as follows: HTN-SE first-round primers: +2548 GATGATGATGATG(T/C)GTGGT and −2859 CC- ATGGGGCTC(T/C)CTCCA; second-round: +2950 TGTTAATGGGGAC(T/A)GTATCTM GCAAAGTTACATTT(T/C)TTCCT and −3108 CCAATACCATAT(C/G)CGACG- second-round: +2770 AQAAAGAAATGGCATTGTTT GC and −3012 CTCAGAACCCTGAT(C/T)CCAT- TC. Because of the hazardous nature of the agent, all steps of the homogenization of autopsy tissue samples and the total RNA extraction and purification were performed under biosafety level 3 conditions. RNA extraction, first-round RT-PCR reactions, and subsequent product DNA gel electrophoresis analysis were performed essentially as described (L. L. Rodriguez, G. J. Letchworth, C. F. Spiropoulou, S. T. Nichol, J. Clin. Microbiol. 31, 2016 (1993)), except the following cycle profile run on a Perkin-Elmer 9600 thermocycler was used: 41°C for 1 h, followed by 40 cycles at 94°C for 40 s, at 38°C for 45 s, and at 72°C for 60 s.

**TECHNICAL COMMENTS**

### Alpha Helix Propensity of Amino Acids

Michael Blaber et al. state that they have found a correlation between changes in stability for substitution mutants of T4 lysozyme at two positions in α helices and the amount of nonpolar surface buried by the substituted side chain when it folds to the native structure (1). If this correlation is real, their work represents a significant advance in our understanding of the structural basis for the different α helix propensities of the amino acids. However, before the validity of the correlation (figure 2 of (1)) can be properly evaluated, several technical issues should be addressed.

For a statistical correlation involving a small data set to have scientific significance, it must be generally true. A provisional test of the proposed correlation for a subset of the amino acid residue (2) substitutions (L, V, I, S, T, K, E, and N) at position 44 can be made from examination of the substitutions at a second site, position 131. Although data are presented for only five substitutions at this position (L, V, I, S, and T), there is no significant correlation between free energy differences (∆G) and buried surface area.

The correlation between the free energy of unfolding and buried surface area for substitutions at position 44 depends on the exclusion of three data points in addition to those for A, G, and P. The exclusion of R because of a crystal contact seems justifiable (though K should perhaps be excluded for the same reason). However, the logic for discarding the critical F and W points [described in note 22 of (1)] is unclear. Blaber et al. appear to argue that, because these two mutants crystallize in space groups that are different from that of the other mutants, the observed trans χ1 angle (which distinguishes these side chains from all others except the wild-type S residue) may be an artifact of the different crystal environments. The fact that the conformation of tryptophan is similar in all four asymmetric environments (and is similar to that of phenylalanine in each of its two asymmetric environments) suggests that this rotamer is not an artifact of crystallization.

Finally, we would caution against the deceptive ease with which statistical correlations can be made. Most of the data presented by Blaber et al. falls into a fairly narrow range of values for both ∆G and for nonpolar surface buried. Nine of 17 residues (excluding G, A, and P) could have, within the experimental error of ±0.1 kcal/mol, the same ∆G value of +0.63 kcal/mol. Even more striking is the narrow range into which most surface area values fall. Particularly noteworthy are the similarities of buried area for A, V, E, T, and S and of calculated areas for extended side chains of Q, R, K, Y, H, and F. This might be expected because, in many cases, the β carbon atom often makes the greatest contribution to buried nonpolar surface. As a consequence of this tight clustering of values within a narrow range, it only takes one or two outlying data points to establish an apparent correlation. This reduction in the number of data points that significantly contribute to a correlation increases the ever-present danger that an apparent correlation might be found by omitting data points from an otherwise random-looking scatterplot. Only when a rigorous and compelling argument is at hand can one, in the search for a better correlation, safely exclude a subset of the data.

While burial of side chain hydrophobic surface may play a role in determining the rank order and magnitude of helix propensity, the correlation reported by Blaber et al. does not convincingly establish that it is the structural basis of amino acid α helix propensity.

**REFERENCES AND NOTES**


2. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

3. 9 July 1993; accepted 14 September 1993

Response: Shortle and Clarke correctly state that if a statistical correlation involving a small data set is to have scientific significance, it must be generally true. We have tested (1) the correlation in question by comparing the area buried on helix formation (as calculated by Richards and Richmond (2)) with experimentally determined scales of helix propensity that are based on substitutions in proteins (1, 3, 4), model peptides (5), host-guest experiments (6), and frequencies observed in known protein structures (7). In every case the correlation was positive, with an average value of 0.49
Alpha helix propensity of amino acids
D Shortle and N Clarke

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