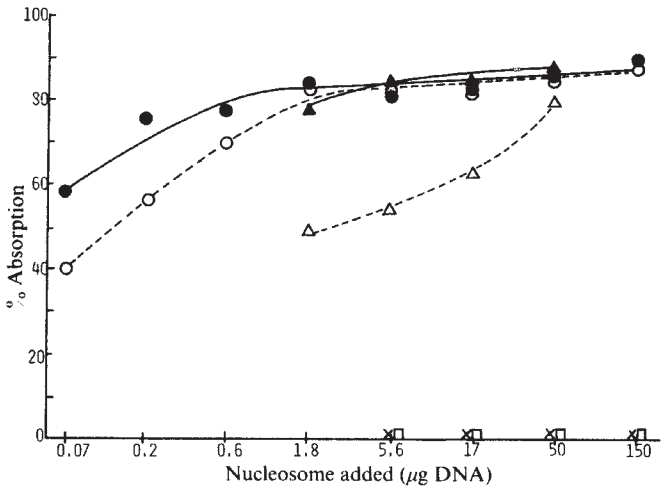

Fig. 2 Specific absorption of rheumatoid factor (RF) from serum Jos and IgM Jos by nucleosomes. IgM was prepared by gel filtration of serum on Sephadex G-200 equilibrated in 0.5 M NaCl, 0.2 M acetate, *p*H 4.0; the first three fractions of the breakthrough peak were pooled, and dialysed against PBS *p*H 7.2. Absorption of RF was effected by mixing the indicated amount of nucleosomes in 80 μl of 0.2 mM EDTA, *p*H 7.0, with 1 ml of serum or IgM diluted in PBS containing 1% bovine serum albumin (PBSA). The solution turned slightly turbid immediately on addition of the nucleosomes. After incubation at room temperature for 2.5 h, the insoluble nucleosomes were sedimented by centrifugation. The supernatants were assayed for RF activity by a solid phase radioimmunoassay[11] using plastic tubes (Falcon 2052) precoated with rabbit IgG. The second antibody was immunoaffinity isolated ^{125}I-labelled rabbit anti-human L-chain antibodies. The incubation time for the first and the second antibody was extended to 18–24 h. The amount of RF absorbed by the nucleosomes was determined from standard curves obtained by making twofold dilutions of the RF-containing sample in PBSA. Absorption of 75% of RF thus corresponds to the radioactive antibody bound by a standard sample diluted fourfold. ○, serum Jos diluted 1/800+nucleosome pool 1; △, serum Jos 1/800+pool 2; ●, IgM Jos (11.5 μg)+pool 1; ▲, IgM Jos (11.5 μg)+pool 2; □, serum Ter 1/200+pool 1; ×, serum Ter 1/200+pool 2.

limited[15] and hydrophobicity may be an essential shared component. A few homogeneous myeloma proteins and Waldenstrom macroglobulins with antigen-binding activity have also been found to possess more than one specificity[15–18]. Thus, RF Jos and similar RF detected in other sera[5,6] may be examples of 'polyfunctional' antibodies.

The crossreacting RF described in the present report emphasises the general problem of extrapolating from the specificity of natural antibodies to the original antigenic stimulus which elicited their production. Moreover, the observations indicate that caution should be exercised in the interpretation of test results where RF are used as reagents, for example in detection of immune complexes.

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1. Hannestad, K. *Clin. exp. Immun.* **2,** 511–529 (1967).
2. Hannestad, K. *Clin. exp. Immun.* **3,** 671–690 (1968).
3. Winchester, R. J., Agnello, V. & Kunkel, H. G. *Clin. exp. Immun.* **6,** 689–706 (1970).
4. Munthe, E. & Natvig, J. B. *Clin. exp. Immun.* **8,** 249–262 (1971).
5. Hannestad, K. & Johannessen, A. *Scand. J. Immun.* **5,** 541–547 (1976).
6. Hannestad, K. *Scand. J. Immun.* **7,** 127–136 (1978).
7. Shaw, B. R., Herman, T. M., Kovacic, R. T., Beaudreau, G. S. & Van Holde, K. E. *Proc. natn. Acad. Sci. U.S.A.* **73,** 505–509 (1976).
8. Thomas, J. D. & Kornberg, R. D. *Proc. natn. Acad. Sci. U.S.A.* **72,** 2626–2630 (1975).
9. Weber, K. & Osborn, M. *J. biol. Chem.* **244,** 4406–4412 (1969).
10. Studier, F. W. *J. molec. Biol.* **79,** 237–248 (1973).
11. Hay, F. C., Nineham, L. J. & Roitt, I. M. *Br. med. J.* **3,** 203–204 (1975).
12. Kornberg, R. D. *A. Rev. Biochem.* **46,** 931–954 (1977).
13. Bauer, K. *Naturwissenschaften* **58,** 364–365 (1971).
14. Johnston, M. F. & Eisen, H. N. *J. Immun.* **117,** 1189–1196 (1976).
15. Michaelides, M. C. & Eisen, H. N. *J. exp. Med.* **140,** 687–702 (1974).
16. Manjula, B. N., Richards, F. & Rosenstein, W. *Immunochemistry* **13,** 929–937 (1976).
17. Bhattacharjee, A. K. & Glaudemans, C. P. J. *J. Immun.* **120,** 411–413 (1978).
18. Tolleshaug, H. & Hannestad, K. *Immunochemistry* **12,** 173–182 (1975).

Hydrophobic character of amino acid residues in globular proteins

PREDICTIVE studies on the secondary structure of globular proteins aimed at locating ordered structural segments have provided little information about spatial orientations or even as to whether residues are exposed or buried. However, the physico-chemical properties[1] of residues can be used to obtain such information. In particular the hydrophobic character[2], is a useful parameter in these studies. The hydrophobic character as defined by the indices given by Tanford[3] and Jones[4] does not reflect hydrophobic environment within protein structures, but we introduce here a new parameter, the 'bulk hydrophobic character' obtained from an analysis of the surrounding hydrophobic environment of amino acid residues in protein crystals. This is a better index of protein hydrophobicity, showing very good correlation with the extent to which residues are buried[5] (correlation coefficient $r = 0.9$) compared with the hydrophobic indices used previously, and it could be used to characterise tertiary structures.

Our study is based on crystallographic data on 14 protein molecules (myoglobin, ribonuclease S, cytochrome *c*, lysozyme, staphyloccocal nuclease, carboxypeptidase A, subtilisin BPN', α-chymotrypsin, carp myogen, cytochrome b_5, apolactate dehydrogenase, trypsin inhibitor complex, cancanavalin A and flavodoxin; see ref. 6 for further details on these proteins). They include representative examples of all known characteristic structural types of proteins defined by Levitt and Chothia[7].

We demonstrated previously[6] that a sphere of 8 Å radius has the required volume for any one residue of a protein molecule for a study of the influence of the surrounding environment. In this report, therefore, we define the 'surrounding hydrophobicity' of a residue as the sum of the hydrophobic indices assigned to the various residues that appear within an 8 Å radius volume in the protein crystal. This parameter is given by:

$$H_j = \sum_{i=1}^{20} L_{i,j} h_i$$

where $L_{i,j}$ is the total number of surrounding residues of the *i*th type associated with the *j*th residue in a given protein, and h_i is the hydrophobicity index for the *i*th residue. All the H values that belong to the same type of residue in the 14 crystals have been grouped together, and the arithmetic average $\langle H \rangle$ for each type has been calculated. These average surrounding hydrophobicities are given in Table 1, together with the corresponding individual hydrophobic indices given by Tanford[3] and Jones[4]. These $\langle H \rangle$ values reflect the preferred hydrophobic environments for the residues and hence their 'bulk hydrophobic character'.

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Table 1 Average surrounding hydrophobicities and the hydrophobicity indices for 20 amino acid residues occurring in globular proteins

Residue	Average surrounding hydrophobicity $\langle H \rangle$ (kcal)	Hydrophobicity index (h_i) (kcal)
Ala	12.97	0.87
Asp	10.85	0.66
Cys	14.63	1.52
Glu	11.89	0.67
Phe	14.00	2.87
Gly	12.43	0.10
His	12.16	0.87
Ile	15.67	3.15
Lys	11.36	1.64
Leu	14.90	2.17
Met	14.39	1.67
Asn	11.42	0.09
Pro	11.37	2.77
Gln	11.76	0.00
Arg	11.72	0.85
Ser	11.23	0.07
Thr	11.69	0.07
Val	15.71	1.87
Trp	13.93	3.77
Tyr	13.42	2.67

In Fig. 1 the individual hydrophobic index (heavy line) is compared with the corresponding preferred surrounding hydrophobicity (thin line) standardised with respect to Gln. Both Fig. 1 and Table 1 show that the surrounding hydrophobicity is not proportional to the corresponding hydrophobic index of the residue. Taking the actual values given in Table 1, the residues can be divided into three groups: Cys, Phe, Ile, Leu, Met, and Val with $\langle H \rangle$ values greater than 14.0 kcal belong to group I. Gly, Ala, His, Trp and Tyr fall into group II with $\langle H \rangle$ values between 12.0 and 14.0 and are thus less hydrophobic than group I residues, and the remaining residues Asp, Glu, Lys, Asn, Pro, Gln, Arg, Ser and Thr with values below 12.0 kcal belong to group III. Val and Ile have the highest surrounding hydrophobicity, with Leu, Cys and Met coming next in order. Though Val is less nonpolar than residues such as Ile, Phe and Trp when defined by thermodynamic transfer measurements according to Tanford, the average surrounding hydrophobicity value suggests that this residue can easily be accommodated inside the molecule. The low value of $\langle H \rangle$ for Trp and Tyr indicate that one polar atom even in a large nonpolar side chain is sufficient to cause a large reduction in the hydrophobic environment. When considering the residues in group III, it is important to note that the surrounding hydrophobicity of Pro lies within the range observed for the polar residues. As the characteristic structure of proline does not allow it to occur in either β strands or helices except at the N terminus, it is restricted to loops that inevitably lie on the surface thus lowering the surrounding hydrophobicity. Of the polar residues Gln, Glu and Arg have higher hydrophobicities, and the preferred environments for the charged residues Asp and Lys are associated with the lowest surrounding hydrophobicities.

Our results show that Val with an hydrophobic index almost half that of Trp, has the highest surrounding hydrophobicity. On the other hand, the surrounding hydrophobicity of Trp is very much reduced even though this residue appears at the top of the hydrophobicity index scale. The same conditions prevail for residues Tyr and Pro which, in spite of high hydrophobic indices, exhibit surrounding hydrophobicities similar to those of more polar residues. These observations suggest that apart from the nonpolarity indicated by the hydrophobic indices of amino acid residue side chains, both their structure and the presence of some polar atoms strongly influence their bulk hydrophobic character and hence their spatial position in protein molecules. Surfaces of globular proteins often contain several nonpolar residues[1,8], mainly group II residues. In our earlier report[9] we pointed out that surface nonpolar residues occur as short distorted α-helical and extended structural domains (one to three residues long) with the backbone polar atoms having high solvent accessibility. The $\langle H \rangle$ parameter therefore characterises the hydrophobic environment preferred by each amino acid residue in a globular protein and we suggest that this specific preference for each residue is an important factor in the folding of a linear chain into a globular shape. We are now studying applications of this parameter to protein folding.

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1. Lee, B. & Richard, F. M. *J. molec. Biol.* **55,** 379–400 (1971).
2. Kauzmann, W. *Adv. Protein. Chem.* **14,** 1–63 (1959).
3. Nozaki, Y. & Tanford, C. *J. biol. Chem.* **246,** 2211–2217 (1971).
4. Jones, David, D. *J. theor. Biol.* **50,** 167–183 (1975).
5. Chothia, C. H. *J. molec. Biol.* **105,** 1–14 (1976).
6. Manavalan, P. & Ponnuswamy, P. K. *Archs Biochem. Biophys.* **184,** 476–487 (1977).
7. Levitt, M. & Chothia, C. H. *Nature* **261,** 552 (1976).
8. Shrake, A. & Rupley, J. A. *J. molec. Biol.* **79,** 351–372 (1975).
9. Manavalan, P., Ponnuswamy, P. K. & Srinivasan, A. R. *Biochem. J.* **167,** 171–182 (1977).

Fig. 1 Comparison of the average bulk hydrophobicity of amino acid residues with their corresponding hydrophobic indices: thin line, average hydrophobicity; broad line, hydrophobic index.