(article in preparation). Moreover, the substitution of total DNA was essentially higher, into DNA samples reassociated at Cot 10^2, for DNA sequences duplicated during the first half of S phase (article in preparation). This finding is consistant with the fact that, at low BrdUrd concentrations, the buoyant density value of DNA synthesized during the first half of S phase is always higher than that of DNA synthesized later (Hartmann and Rode[29] and not published results). An explanation lies in the fact that the synchronization procedure used - including a 4-day cell starvation - generates some major metabolic disturbances, such as an unbalanced nucleotidic pool or quantitative variations of some of the enzymes involved in DNA synthesis, which would favor a preferential incorporation of BrdUrd during a short period (several hours) following release of mitosis. From another point of view, Kuebbing and Werner[30] have shown that exogenous dThd added to Hela cells grown in thymidine-free medium is incorporated into DNA almost immediately at full specific activity, blocking any further incorporation of de novo synthesized thymidine nucleotides. These experiments suggest a compartmentation of intracellular salvage and de novo thymidine nucleotide pools. Furthermore, Mattern and Painter[31] have found that, when moderately repeated sequences were replicated, exogenous dThd was incorporated at a faster initial rate - particularly during early S phase - than when highly repeated and unique sequences were replicated. As, in our system, exogenous BrdUrd can be only incorporated by the salvage pathway, the over-substitution of rDNA, relative to total DNA, observed at low concentration in culture medium, may then be explained by the association of the 2 above-mentioned metabolic processes[30,31]. In these conditions, the under-substitution of rDNA observed with higher BrdUrd concentrations is far from clear. The low A+T content of rDNA could be involved in this phenomenon, as well as a feedback control of the synthesis of the BrdUrd triphosphate pool, induced by a much greater concentration into the salvage nucleotidic pool. Finally, even if our results are concerned with an intermediate and well-defined DNA sequence, it is quite obvious that they cannot be directly extended to the whole of the intermediate cellular DNA.

1 This work was supported in part by grants from the Délégation Générale à la Recherche Scientifique et Technique (contract No. 74419) and from the Institut National de la Santé et de la Recherche Médicale (C.R.L. No. 802011). The authors wish to thank M.T. Giordano for technical assistance.
2 Present address: Laboratoire de Biologie Moléculaire Végétale, Bât. 430, Université Paris XI, F-91405 Orsay (France).
3 Present address: I.N.S.E.R.M., U 186, Institut Pasteur, 16, Rue C. Guérin, BP 3415, F-59819 Lille Cedex (France).
4 Rutter, W.J., Pictet, R.L., and Morris, P.W., A. Rev. Biochem. *42* (1973) 601.
5 Schwartz, S.A., and Kirsten, W.H., Proc. natl Acad. Sci. USA *71* (1974) 3570.
6 Strom, C.M., and Dorfman, A., Proc. natl Acad. Sci. USA *73* (1976) 1019.
7 Durante, J., Geri, C., Nuti-Ronchi, V., Martini, G., Guillé, E., Grisvard, J., Giorgi, L., Parenti, R., and Buiatti, M., Cell Diff. *6* (1977) 53.
8 Monier, F., Guespin, J., and Felenbok, B., Exp. Cell Res. *107* (1977) 397.
9 Sinclair, J.H., and Brown, D.D., Biochemistry *10* (1971) 2761.
10 Merits, I., Schultz, W., and Overby, R.L., Archs Biochem. Biophys. *115* (1966) 197.
11 Eden, F.C., and Hendrick, J.P., Biochemistry *17* (1978) 5838.
12 Temin, H., and Rubin, H.M., Virology *6* (1958) 669.
13 Goldé, A., Aghion, J., and Villaudy, J., Intervirology *1* (1973) 242.
14 Hughes, S.H., Shank, P.R., Spector, D.H., Kung, H.J., Bishop, J.M., Varmus, H.E., Vogt, P.K., and Breitman, M.L., Cell *15* (1978) 1397.
15 Rode, A., de Taisne, C., and Hartmann, C., Biochem. biophys. Res. Commun. *103* (1981) 1343.
16 Gillespie, D., and Spiegelman S., J. molec. Biol. *12* (1965) 829.
17 Schildkraut, C.L., Marmur, J., and Doty, P., J. molec. Biol. *4* (1962) 430.
18 Luk, D.C., and Bick, M.D., Analyt. Biochem. *77* (1977) 346.
19 Amaldi, F., Giacomoni, D., and Zito-Bignami, R., Eur. J. Biochem. *11* (1969) 419.
20 Stambrook, P.J., J. molec. Biol. *82* (1974) 303.
21 Balazs, I., and Schildkraut, C.L., Exp. Cell Res. *101* (1976) 307.
22 Zellweger, A., Ryser, U., and Braun, R., J. molec. Biol. *64* (1972) 681.
23 Giacomoni, D., and Finkel, D., J. molec. Biol. *70* (1972) 725.
24 Newlon, C.S., Sonenshein, G.E., and Holt, C.E., Biochemistry *12* (1973) 2398.
25 Balazs, I., and Schildkraut, C.L., J. molec. Biol. *57* (1971) 153.
26 Gimmler, G.M., and Schweitzer, E., Biochem. biophys. Res. Commun. *46* (1972) 143.
27 Epner, E., Rifkind, R.A., and Marks, P.A., Proc. natl Acad. Sci. USA *78* (1981) 3058.
28 de Taisne, C., Doctoral thesis. University of Paris VI, Paris 1982.
29 Hartmann, C., and Rode, A., C. r. Acad. Sci. Paris *295* (1982) 69.
30 Kuebbing, D., and Werner, R., Proc. natl Acad. Sci. USA *72* (1975) 3333.
31 Mattern, M.R., and Painter, R.B., Exp. Cell Res. *111* (1978) 167.

0014-4754/83/101134-03$1.50+0.20/0

A semi-empirical formula for the polarizability of the naturally-occurring amino acids based on their side chain packing volumes

D.A. Deranleau

Theodor Kocher Institute, University of Bern, CH-3012 Bern (Switzerland), May 2, 1983

Summary. A linear relationship between the polarizability of the side chains of the naturally-occurring amino acids and their side-chain packing volumes in protein crystals is demonstrated.

The polarizability of atoms and molecules is important in the analysis of a wide variety of physical and chemical problems, for example, in light scattering, electric dipole moment calculations, and the evaluation of van der Waals forces. The polarizability is usually determined from refractive index measurements by means of the Lorentz-Lorenz equation, and for atoms and simple small molecules it is of the order of the molecular volume. A similar consideration

should apply to larger and more complex molecules, particularly to those which fall in a given class, like the amino acids. Here I show that such a relationship exists for the naturally-occurring amino acids, and that it is suprisingly linear when the molecular volumes are defined as those which each side chain occupies (on the average) in tight packing in protein crystals.

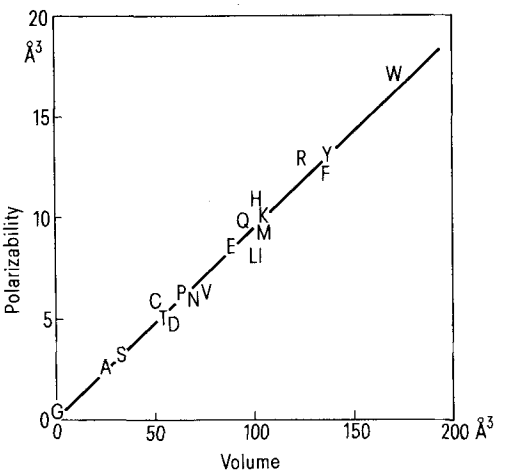
The molar refractions of the individual amino acid side chains were obtained by summing the appropriate group molar refractions, which were taken from the extensive tables of substituent coefficients for structure-activity correlations published by Hansch et al.[1]. The molar refractions were converted to polarizabilities by means of the Lorentz-Lorenz equation, with the usual assumption that the optical frequencies on which the molar refractions are based correspond to infinite frequency. The side chain volumes for the amino acids were calculated from the whole-residue packing volumes (the average volume occupied by a given residue in the interior of a protein)[2,3] by subtracting the packing volume of glycine from each of the published values. A plot of the calculated polarizabilities of the amino acids against their corresponding side chain packing volumes is shown in the figure. The data are best described by the straight line:

$$\alpha = 0.0726 + 0.0945\ V \simeq 0.0945\ V \qquad (1)$$

with a correlation coefficient of 0.96. The side chain volumes are 25 Å^3 or more, and neglecting the small first term leads to a maximum 3% discrepancy (alanine) as compared with the complete equation, so that within the limits of the treatment used, the polarizability of an amino acid side chain is directly proportional to its packing volume.

Among the possible areas in which the relation (1) might find application is in the ab initio calculation of 3-dimensional protein structures. The number of potential structures which must be evaluated in order to find the global energy minimum which corresponds to the most probable structure is enormous if the calculations are carried out on an atom-by-atom basis, and a considerable simplification of the problem is afforded by methods in which certain groups of atoms or whole side chains are treated as single entities[4]. The best sources of the van der Waals energy parameters for protein folding calculations appear to be those that use geometries based on single crystal data[5], so that semi-empirical relationships like the one discussed above may find some use in this application.

Similar linear relationships have been discovered between the hydrophobicity of the amino acid side chains and their crystal packing volumes[6], but here the data appear to fall into 2 classes, depending on whether a potential hydrogen-bonding heteroatom is present or not. Clearly van der Waals interactions (dispersion forces) between side chains are responsible for a significant fraction of the side chain contribution to so-called hydrophobic forces, and the linearity of 'hydrophobicity' with side chain packing volume is not surprising in the light of the linearity of the side chain polarizability with packing volume.



Polarizability of the amino acid side chains as a function of their side chain packing volumes. Amino acids included in the fitted curve are: Trp (W), Tyr (Y), Phe (F), Arg (R), Lys (K), Met (M), Ile (I), Leu (L), His (H), Gln (Q), Glu (E), Val (V), Asn (N), Pro (P), Asp (D), Thr (T), Cys (C), Ser (S), Ala (A), Gly (G).

1 Hansch, C., Leo, A., Ungar, S.H., Kim, K.H., Nikaitani, D., and Lien, E.J., J. med. Chem. *16* (1973) 1207.
2 Chothia, C., Nature, Lond. *254* (1975) 304.
3 Teller, D.C., Nature, Lond. *260* (1976) 729.
4 Levitt, M., J. molec. Biol. *104* (1976) 59.
5 Levitt, M., A. Rev. Biophys. Bioengng *11* (1982) 251.
6 Chothia, C., Nature, Lond. *248* (1974) 338.

0014-4754/83/101136-02$1.50 + 0.20/0

Mixed lymphocyte reaction in human monoclonal gammopathies[1]

A.L. Carmagnola, M. Boccadoro and A. Pileri[2]

Chair of Haematology, University of Turin, San Giovanni Hospital, Via Genova 3, 1-10126 Turin (Italy), May 25, 1982

Summary. Ten multiple myeloma (MM) and 5 monoclonal gammopathies of undetermined significance (MGUS) were studied. The mixed lymphocyte reaction (MLR) proliferative response was of the same order of magnitude in MM and in MGUS as in normal controls. Normal results were obtained when pathological lymphocytes were used as either responding or stimulating cells. The addition of monoclonal anti-Ia antibodies completely abrogates the proliferative response as in healthy individuals. These results suggest that the immunological mechanisms supposed to be important in MLR are functionally normal in MM and in MGUS, although both the B and T cell lineages are involved in monoclonal gammopathies.

In multiple myeloma (MM) and in monoclonal gammopathy of undetermined significance (MGUS), a great number of lymphocytes displaying the same idiotypic specificities was demonstrated by using antisera directed against the idiotypic specificities of the M component[3-8]. A lymphocyte subset, rosetting with sheep red blood cells (SRBC), devoid