DSS AS AN ACTIVATOR OF CARBOXYPEPTIDASE A

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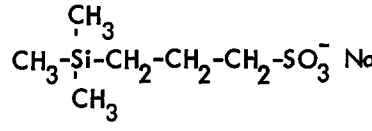
Received May 27, 1969

A generally accepted internal reference for NMR studies of aqueous solutions is sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), [1-3]



This material has been used extensively as an internal reference for studies of biological molecules, including enzymes and proteins. [4-5] One of the requirements of an internal reference, which is dissolved directly in the solution under investigation, is the absence of interaction with any one of the components of the solution. Thus, having the intention of using DSS as an internal reference in our n.m.r. studies of carboxypeptidase A, [6] a possible effect of DSS on the enzyme activity was first checked. As a result, we wish to report that DSS binds strongly to carboxypeptidase A and enhances its peptidase activity. The maximum enhancement is dependent on substrate concentration and more than fourfold enhancement was obtained at 10^{-4}M substrate concentration. Activation for two substrate concentrations is illustrated in Fig. 1.

In a preliminary consideration, it was found that K_A, the binding constant of the activator to the enzyme, could be determined by plotting $v_o / (v - v_o)$ vs. $1 / [A]$ where v and v_o are the initial reaction

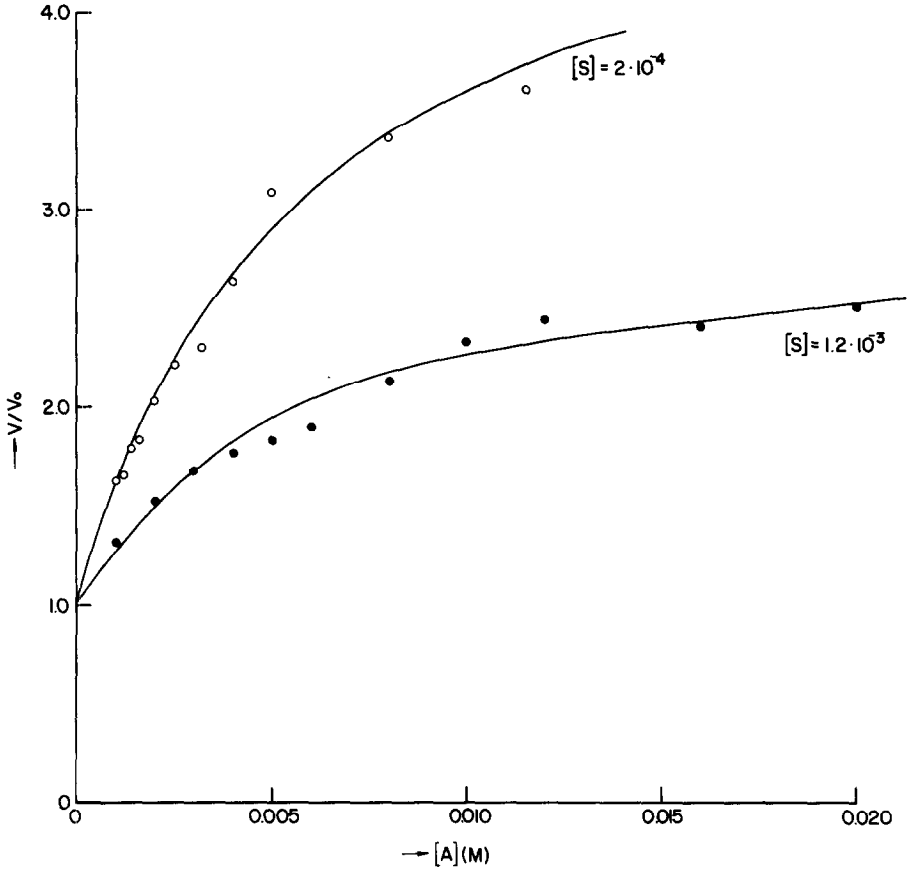


FIG. 1:

Relative hydrolysis rates of hippuryl-L-phenylanine as a function of DSS concentration, [A]. The reaction was followed spectrometrically at 25°C.

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rates in the presence and absence of the activator, A, respectively. This provides a linear plot, and for a sufficiently low substrate concentration its X-axis intercept is equal to K_A. This could be shown to hold true for a variety of conceivable models of substrate and activator binding.

In our experiments, each with a constant substrate concentration, hippuryl-L-phenylalanine and a varied amount of the activator DSS, a straight line was obtained, using the above plot. This is illustrated

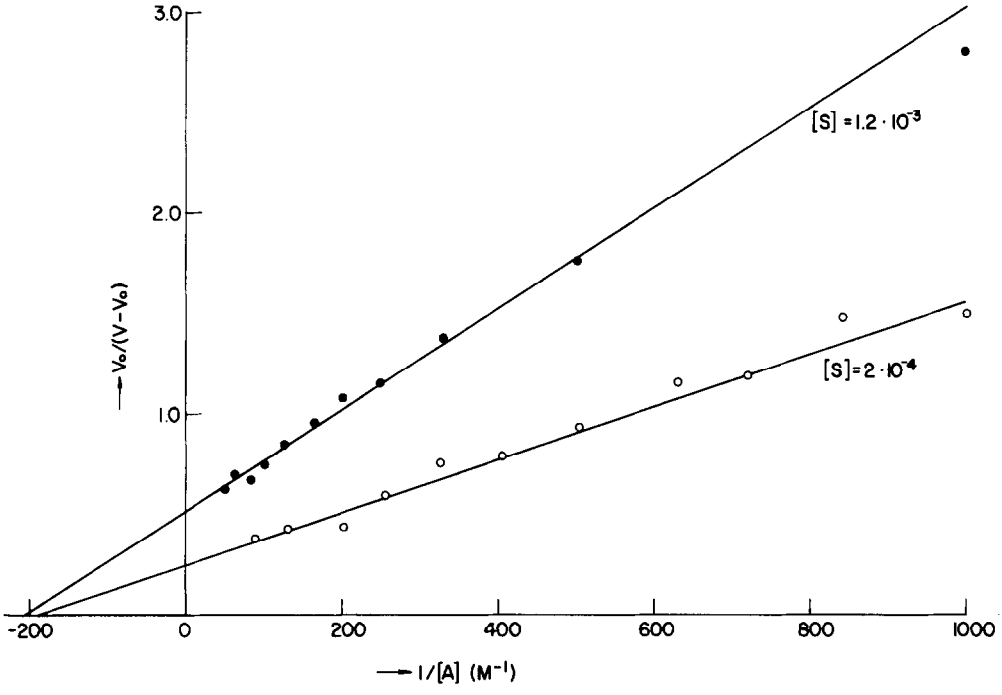

FIG. 2:

A linear plot of $v_o/(v-v_o)$ vs. reciprocal DSS concentration [A]; using the results given in Fig. 1.

in Fig. 2 using the results given in Fig. 1. The best straight line was fitted by the method of least squares,[7] and the X-intercept obtained therefrom. All the measurements were taken at constant temperature $25.0 \pm 0.1^{\circ}C$. The initial slopes of the spectrophotometric trace of the reaction at 254 mμ were taken as the experimental reaction rates.[8]

The value of this intercept was found to be $(2.0 \pm 0.4) \times 10^{2} M^{-1}$ and was approximately independent of substrate concentration in the range of 1.0×10^{-4} - $1.2 \times 10^{-3} M$. This makes extrapolation to zero substrate concentration unnecessary, and the above value can be taken as the binding constant of the activator, K_A. Such a value for K_A indicates that half of the enzyme present is bound to DSS at a concentration of about $5.0 \times 10^{-3} M$ DSS.

A complete kinetic analysis is complicated because of substrate activation which occurs with hippuryl-L-phenylalanine used in this work.[9] Further work on this study is in progress.

Although DSS is analogous to some of the known activators and inhibitors[10,11] in having hydrophobic and polar groups, it is chemically different from all of them. Its distinct structure and its high binding constant make DSS adequate for X-ray investigation of the location of the activator molecule on the enzyme. The difference map between the enzyme and the enzyme-DSS complex might be more easily interpreted than that of known activators since the effect of DSS on the difference map can be distinguished from that of a conformational change, such as observed by Reeke et al. upon binding glycyl-tyrosine to carboxypeptidase A.[12] The possibility of confusion might be eliminated in view of the dissimilarity of DSS to any of the protein components.

Returning to the main point of this report, it must be stressed that when using DSS as an internal reference in protein research by n.m.r. one should be aware of its possible interaction with the protein.

REFERENCES.

1. G.V.D. Tiers and R.I. Coon, J. Org. Chem., 26, 2097 (1961).
2. J.R. Dyer, "Application of Absorption Spectroscopy of Organic Compounds", Prentice-Hall, 1965, p.64-65.
3. J.W. Emsley, J. Feeney and L.H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy", Pergamon Press, 1965, p. 265-266.
4. C.C. McDonald and W.D. Phillips, J.Am. Chem.Soc., 89, 6332 (1967).
5. K. Wüthrich, R.G. Shulman and J. Peisach, Proc. Natl. Acad. Sci. U.S., 60, 373 (1968).
6. For an earlier work on this subject, see: G. Navon, R.G. Shulman, B.J. Wyluda and T. Yamane, Proc. Natl. Acad. Sci., 60,86 (1968).

7. W.W. Cleland, in "Advances in Enzymology", F.F. Nord (ed.), Interscience Publishers, 1967, p. 1-30.

8. J.E. Folk and E.Q. Schirmer, J. Biol. Chem., 238, 3884 (1963)

9. R.C. Davis, J.F. Riordan, D.S. Auld and B.L. Vallee, Biochemistry 7, 1090 (1968).

10. R.C. Davis, D.S. Auld and B.L. Vallee, Biochem. Biophys. Res. Commun. 31, 628 (1968).

11. I. Schechter and E. Dascal, Israel J. Chem., 6, 118p (1968).

12. G.N. Reeke, J.A. Hartsuk, M.L. Ludwig, F.A. Quiocho, T.A. Steitz and W.N. Lipscomb, Proc. Natl. Acad. Sci. U.S., 58, 2220 (1967).