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Dear Jacques:

Patlak has finally given me the results of the computer analysis of the  $I_2$  formation data. He has analysed both sets of experiments. In some ways they were disappointing. The first reason was that he could not separate out the individual rate constants in our model but could solve only for combinations of them which are difficult to interpret meaningfully in a physical sense. If you go back to our most general model, you will find what the constants represent. Using those constants, Patlak has given us the following results:

Constants	1° Series	2° Series
$k_{12}K_T$	10038.2	11287.1
$[(k_{11}/k_{10}) + (k_{12}/k_{10}) + (k_{12}/k_1)]$	97.032	106.689
$\frac{k_2}{k_1} [(k_{11}/k_{10}) + (k_{12}/k_{10})]$	201.825	148.289

You will note that  $(k_2/k_1)$  and  $(k_{11}/k_{10})$  are contained in the combinations of constants; they represent the  $K_m$  values for the two sites for  $I^-$ . We would, therefore, like to know them, but

we have not yet figured out a way to separate them out from other constants and solve for their exact values. It is easy to get  $k_{12}$  because  $E_T$  is total enzyme content, a variable which you know but did not send us with the data.  $k_{12}$  then represents essentially the theoretical maximum turnover number of the enzyme in units of  $\Delta$  Absorbance/unit time/mg of enzyme.

The second reason for disappointment is that the computer program also estimates the standard errors and reliability of the values for the combinations of constants which it has determined. Unfortunately, the reliability is not high. The reason for the poor reliability is that all the values for the reaction velocities were in the range of being almost proportional to iodide concentration. If you plot velocity vs. iodide concentration, you will note that it is almost a straight line. The model predicts a sigmoid curve and to test it properly, it is necessary to extend the range to see whether the curve really bends at both extremes of iodide concentration. If we had such a range, it would greatly improve the reliability of the constants. If the constants which we have thus far are anywhere near their real values, then we estimate that to include sigmoidicity at both ends of the curve, you will have to extend the iodide concentration down to  $1 \times 10^{-4}$  M or below and up to at least  $100 \times 10^{-4}$  M. We estimate that saturation of the enzyme would not occur until  $300-400 \times 10^{-4}$  M.

I know that there are problems in carrying out the assays at very high and very low levels of iodide concentration. Perhaps, if the conditions are such that the reaction rate is linear with enzyme concentration, it would help to vary the peroxidase concentrations at the extremes to bring the reaction velocities in the range where they can be measured. The velocities could then be normalized by reporting them as velocity per mg of enzyme. That would not alter our kinetic analyses.

Let me know what you think. Myself, I am still troubled by getting only combinations of constants rather than the individual constants themselves, or at least the ratios of those constants which would give us the  $K_m$  values which we want. Perhaps, we will be able to figure out a way to get these out. In the meantime, let me know what you think we should do about it. I suppose, if it is not too much trouble, it would still be worth doing the experiments which extend the range of the curve.

We were very happy to hear that you will be participating in a symposium at the Endocrine Congress next summer and that you will

be visiting here again. You would be most welcome to stay at our home during the meetings. Things are very much the same at our home. The family is fine; Ken is doing well at school and Ann is busy applying to colleges for next year and awaiting the acceptance or rejection notices.

Regards to Paulette and the children and also to everyone at the Lab. It was very nice to see them all again, and I am looking forward to the next opportunity.

Yours sincerely,

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