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OPEN UNIVERSITY PRODUCTION CENTRE

COURSE: S342

PROGRAMME: TV3 REACTION MECHANISMS I
Hydrolysis

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EXECUTIVE PRODUCER .....Andrew Crilly

PRODUCER/DIRECTOR .....Barrie Whatley

PRODUCTION ASSISTANTS.....Kim Leadbitter
Christina Clements

SPEAKER .....Dr. Peter Taylor

LOCATION CAMERA UNIT .....Tim Konewko
Gordon Blockley

Sound Martin Ward
Mike Heald

COMPUTER GRAPHICS.....Philip Butcher

GRAPHIC DESIGN.....Dick Laing
Kim Williams

LOCATION:

Venables Building OU

S342 Opening Titles
Physical Chemistry
Reaction Mechanisms I
Hydrolysis

Music
Parry Music Library 9
Dur:12"

Dr. Peter Taylor V/O
Open University
WS food on table

Carboxylic acids and their derivatives are one of the most important classes of organic compound.

The oils and fats you cook with are esters.

The proteins in meat, eggs and other food sources are excellent examples of amides.

One of the key reactions of these compounds is their reaction with water - hydrolysis.

The manufacture of soap involves an ester hydrolysis: the breakdown of proteins in the stomach and intestine represents an example of amide hydrolysis. Clearly this is an important type of reaction and it's not surprising that it's one of the most widely studied in chemistry.

But most carboxylic acids are remarkably stable. /Both in industry and in nature rapid hydrolysis requires the use of a catalyst - the body uses enzymes for this purpose. From the chemist's molecular viewpoint, one of the most interesting aspects of this and indeed any reaction, is the reaction mechanism,

Cut to MS Dr. Peter Taylor

the detailed molecular changes and the various stages involved in the reaction pathway.

In this and the next programme I'll give you a blow by blow account of how such a mechanism is elucidated and the reaction we'll study is the hydrolysis of an ester, para nitrophenyl acetate or PNPA for short.

Cut to Video Rostrum PNPA V/O Peter Taylor

This hydrolysis gives para nitrophenol and acetic acid. This reaction is catalysed by a number of species but the catalyst I'll use is N-methylimidazole. This type of system has been well studied since the imidazole structure is part of the active site of a large number of enzymes that catalyse ester and amide hydrolysis.

Cut to MS Peter Taylor

A mechanism is usually determined by inference from all the known facts about a reaction. But it's often the kinetics that provides the greatest insight into the reaction mechanism. So we'll want to start by establishing the experimental rate equation.

Now as you know, before we do any kinetic experiments, the first thing we want to do is to determine the stoichiometry of the reaction. /For this reaction using a combination of infrared and ultraviolet spectroscopic techniques, it's been confirmed that

Cut to Video Rostrum V/O Peter Taylor

this stoichiometric equation applies throughout the whole course of the reaction.

Cut to MS Peter Taylor

So it exhibits time independent stoichiometry, now of course this does not imply that there are no intermediates in the reaction - just that within the accuracy of the analysis they can't be detected. The next step is to decide which factors effect the rate of reaction, so that we can plan our experiments to give the most useful data. In proposing a possible rate equation, we need to consider what species can effect the rate of reaction.

Cut to Chyron seq. 1
V/O Peter Taylor

Obviously we should include the reactants, water and para nitrophenyl acetate. Since the catalyst effects the rate the concentration of N methyl imidazole-NMI-should also be included. And we'll give each of these species a partial order of reaction alpha, beta, or gamma.

Cut to CU Peter Taylor

Now in practise water is not only a reactant but it's also a solvent, so it's concentration is present in great excess, compared to that of para nitrophenyl acetate and does not change significantly during the reaction.

Cut to Chyron seq. 2
V/O Peter Taylor

And this poses us with a problem, since we can't effectively vary the concentration of water we can't determine the partial order alpha directly by experiment. Nevertheless, since the water concentration is effectively constant, we can combine this term with the rate constant to give a modified rate equation.

Cut to MS Peter Taylor

Usually at this point, when we need to determine the partial order with respect to more than one species, we use an isolation technique that is we keep all the concentrations but one in excess and so isolate the dependence of the rate of the reaction on the concentration of that one species. But here we don't have to resort to this, because N methyl imidazole is a catalyst it isn't consumed so it's concentration doesn't change during the reaction. This means that for a single kinetic experiment this term in the experimental rate equation is constant, so we can simplify the rate equation. /Where K - R double dash incorporates K - R dash and the fixed concentration term for N-methyl imidazole. Whilst this makes the calculation of gamma a little trickier it does simplify the

Cut to Chyron seq. 3
V/O Peter Taylor

determination of beta. Now we only have to perform an experiment to determine how the concentration of para nitrophenyl acetate varies with time and beta can be calculated using the differential or integration method. /So now we come to the practical aspects of this experiment. How do we monitor the change in concentration? Well one of the reasons why I chose to use para nitrophenyl acetate rather than a simple ester such as ethyl acetate was because compounds that contain the para nitrophenyl group absorb light in the UV and visable region. This means that we can continuously monitor the change in concentration with time of such species using a UV visable spectrophotometer.

Cut to MCU Peter Taylor

Laboratory Seq.1
Brandon Cook
V/O Peter Taylor

First we shall monitor the reaction over a wide range of wavelengths. Since both the reactant para nitrophenyl acetate and the product paranitrophenol will have characteristic but different absorption spectra in the UV visable region, the spectrum will change during the course of the reaction. The peak which occurs first around 270 nanometres is caused by th reactant - para nitrophenyl acetate.

Lab. seq. /cont.

But as the reaction proceeds this decreases as another peak at 400 nanometres increases due to the product para nitrophenol, which at this pH exists predominently in its ionised form, the para nitro phenolate ion. For a quantitative study we need to choose a single wavelength where the absorbance is proportional to the concentration of just one species. This turns out to be around 400 nanometers where the absorption is due only to the product - para nitrophenolate - using the Beer Lambert law we can relate the absorbance A, at a given wavelength, to the concentration C of the absorbing species. Eba is the molar absorption coefficient and l is the path length through the solution.

So by measuring the absorbance at 400 nanometres we can study how the concentration of the product varies with time.

Let's now look in detail at how such an experiment is performed. 2.5 cubic= centimetnes of a solution containing a known concentration of the N-methyl imidazole catalyst is transferred to each of two identical cells - one to be used as the sample and the other to serve as a blank in the reference/

/beam.

Lab. seq. /cont.

The cells are placed in the cell block and left there for about 15 minutes to equilibriate to the desired temp. In this case the cell block is maintained at a constant temperature of 25°C by water circulating from a thermostated bath.

In the meantime the wavelengh is set, and the instrument zeroed.

To start the reaction a known amount of a stock solution of para nitrophenylacetate is added to the sample cell shaken well to ensure efficient mixing, and replaced in the sample compartment, and the recording started as quickly as possible.

The increase in absorbance indicates the formation of the product. After a while the rate of increase in absorbance is decreased due to the reduced concentration of reactant available to form the product.

Eventually the absorbance curve flattens off towards a constant value as the reaction nears completion.

Cut to MS Peter Taylor

Well now that we have the experimental rate data, we can use the integration method to determine beta. We use the integration method because it's the most convenient since the data could be directly feed in to the integrated rate equations to find the best fit.

Chyron seq. 4.
V/O Peter Taylor

This reaction is most likely first or second order. /So we start by seeing if the data fits either of these integrated rate equations, each of which of course are equations for straight lines. But for this we need to know how the concentration of the reactant para nitrophenol acetate varies with time. /Whereas our experimental data shows how the absorbance of the product varies with time. Fortunately there are a number of simple ways of achieving this conversion. For example, knowing the cell pathway's length and the molecular extiction coefficient for para nitrophenolate we use the Beer Lambert law to convert the data from absorbance to concentration of para nitrophenolate ion.

Computer Graphics Seq. 1
V/O Peter Taylor

Cut to CU Peter Taylor

From the stoichiometry we know that one molecule of paranitrophenylacetate gives one molecule of the product paranitrophenylate ion, and also that the reaction exhibits time independent stoichiometry. /So its a simple matter to convert this data for the rise in para nitrophenolate ion with time to a curve for the decrease in the concentration of paranitrophenol acetate the reactant.

Computer Graphics Seq. 2
V/O Peter Taylor

Now that the data is in the right form we can see if it fits either the first or second order integrated rate equations, remembering to ensure that the data extends to over 50% reaction. For the first order we need to plot natural log PNPA concentration against time and for the second order the reciprocal of the PNPA concentration against time. Clearly the data fits the first order plot giving a good straight line.

This indicates that the reaction is first order with respect to para nitrophenyl acetate./So beta is 1.

Cut to Chyron seq. 5
V/O Peter Taylor

Having determined beta our next task is to determine gamma, the partial order with respect to N-methylimidazole.

Cut to MCU Peter Taylor

Now you'll remember that we can't do this directly from experiment, because being a catalyst the concentration of N-methylimidazole does not change during the reaction. But there is a way round this./Using the integrated rate equation we have just produced we can calculate K R double dashed from the slope. And as shown in the line above K R double dashed is a function of the concentration of N-methylimidazole.

Cut to Chyron seq. 6
V/O Peter Taylor

Taking logs of this gives the equation of a straight line of the slope gamma, so if we repeat the experiment the varying concentrations of N-methyl imidazole and plot the logs of the resulting values K-R double dash against the log of the N-methyl imidazole concentrations, this is what we get. And this gives us a straight line of slope 1. So gamma is 1 and the reaction is first order with respect to N-methyl imidazole.

Cut to Computer Animation 3 - V/O Peter Taylor

Chyron seq. 7

Having determined that the reaction is first order with respect to the concentrations of not only para nitrophenol acetate but also N-methyl imidazole, we can now re-write our experimental rate equation.

Remember that while K - R dash could well be some function of the concentration of water there is no way we can determine this since we cannot vary the concentration of the solvent.

Cut to MCU Peter Taylor

Nevertheless we can now turn to consider what insight this experimental rate equation provides into the mechanism of the reaction.

Chyron seq. 8

If this were an elementary reaction, then the order with respect to water which after all is one of the reactants would have to be at least 1 -

and the experimental rate equation would be third order. Well think about what this means - it means that for the reaction to occur you'd need a ter molecular collision - and that's very unlikely. So this points to a composite reaction, but what is it's mechanism?

Cut to MCU Peter Taylor

Unfortunately the kinetics of this reaction give us little further information on the exact form of this mechanism, other than that any mechanism that we propose should generate a chemical rate equation in agreement with its experimental counter part. It's here that the chemist has to do a little bit of lateral thinking and examine evidence from other sources.

Cut to Video Rostrum 3

This is imidazole it also catalyses the hydrolysis, and since it has a similar structure to N-methyl imidazole we might expect both reactions to proceed by a similar mechanisms.

So what happens when we repeat the experiment with imidazole?

Cut to Lab seq. 2
Brandon Cook
V/O Peter Taylor

Well with low concentrations the results are very similar leading to the same form of experimental rate equation, but when we use a high concentration of imidazole, we find that the reaction no longer exhibits time independent stoichiometry.

Monitoring the absorbance at 245 nanometres we get a peak indicating the build up and decay of an intermediate. This has been shown to be N-acyl imidazole, and so we can now propose a mechanism, involving the formation and decay of this intermediate. Reasoning by analogy we can propose asimilar mechanism for the N-methyl imidazole catalysed reaction. That is a two step mechanism involving an N-acyl, N-methyl imidazole intermediate. And of course you won't be surprised to learn that this mechanism is consistant with the experimental rate equation.

Cut to Video Rostrum 4.

Cut to MS Peter Taylor

Well I've given the derivation of this chemical rate equation in the broadcast notes and there you will see that to produce it I've used the steady state assumption, that is I have assumed that the rate of change in the concentration of the intermediate with time is negligible and can be approximated to zero.

Now let's spend a few minutes examining the assumption in a little more detail. And to do this we use a simplified two step system.

Computer Graphics/Anima. 5 V/O Peter Taylor

A goes to B goes to C, with rate constants k_1 and k_2. Now I'm going to get the computer to plot the variation in the concentrations of A,B and C with time for various ratios of k_1 and k_2.

Cut to CU Peter Taylor

Of course the actual shape of these time concentration curves would depend on the actual values of the rate constants, but for a given ratio of rate constants the shapes would be the same irrespective of their absolute magnitudes - only the time scale over which the reaction occurs will vary. Let's have a look

Computer Graphics/Anima. 6 V/O Peter Taylor

what happens when K_1 is much larger than K_2.

So the first reaction is intrinsically much faster than the second, and A is quickly converted to B, but B only slowly forms C.

Clearly the intermediate B builds up in this reaction and the reaction does not exhibit time independent stoichiometry.

Now let's see what happens when the rate constants are changed to a similar size.

The intermediate still builds up but to a lesser extent.

Looking at these curves you may realise that this is the sort of behaviour that we observed with the imidazole catalyst.

Now what do you think we'll get when K_2 becomes much larger than K_1? As the second reaction becomes steadily faster than the first the extent to which the intermediate builds up decreases. Eventually when K_2 is much larger than K_1 the concentration of the intermediate becomes negligible. This is the steady state region where the rate of formation B is equal to its rate of decay.

So now the reaction does exhibit time independent stoichiometry since throughout the reaction the sum of the concentrations of the reactants and products A and C is effectively equal to the initial reactant concentration. This is of course the behaviour we found using the N-methyl imidazole catalyst.

Expanding the concentration axis you can see that the concentration does vary, but the change is small indeed, and so the steady state approximation which assumes the rate of change in the concentration of B with time is zero -introduces very little error.

But this is not strictly true at the very beginning of the reaction - the steady state takes some time to be achieved.

Video Rostrum 5.
V/O Peter Taylor

Since the N-methyl imidazole catalysed hydrolysis of para nitrophenyl acetate probably occurs by this mechanism, yet still exhibits time independent stoichiometry, the concentration of the intermediate must be very low. It's a reactive intermediate.

Cut to MS Peter Taylor

So we have found out a great deal about the mechanism of N-methylimidazole and imidazole catalysed hydrolysis of para nitrophenyl acetate, and in the next programme we consider how we tackle this problem for enzyme catalysed hydrolysis.

Music Dur

Chyron roller
over CAVITY animation

The speaker was
Dr. Peter Taylor

Laboratory Assistance
Brandon Cook

Production Assistants
Kim Leadbitter
Christina Clements

Graphic Design
Dick Laing
Kim Williams

Computer Animations
Philip Butcher

Location Camera
Tim Konewko
Gordon Blockley
Sound
Martin Ward
Mike Heald

Series Producer
Barrie Whatley

Executive Producer
Andrew Crilly

39"

A production for the OU etc