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Effects of Ionization of *P*-Nitrocatechol Sulfate on its Behavior as a Substrate for Arylsulfatases*

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Evidence is presented showing that arylsulfatases reacting at acidic pH are more active towards *p*-nitrocatechol sulfate substrate, while the reverse is true for the enzymes having alkaline pH_{max}. An explanation of this phenomenon is suggested based on the ionization of *p*-nitrocatechol sulfate ($pK_a = 6.4$) and a much higher reactivity of sulfatases with the acidic form of the substrate.

Arysulfatases are enzymes which catalyze the hydrolysis of arylsulfates, as shown in Eq. (1). The most commonly used substrates to test arylsulfatase activity are *p*-nitro-

$$\text{Ar-OSO}_3^- + \text{H}_2\text{O} \xrightarrow{\text{Arylsulfatase}} \text{ArOH} + \text{HSO}_3^- \qquad (1)$$

phenyl sulfate (*p*-NPS) and *p*-nitrocatechol sulfate (*p*-NCS).

It has been observed that sulfatases which react more readily with *p*-NPS have pH maxima more alkaline than those for which the preferred substrate is *p*-NCS. While these data may not be completely exhaustive, we are not aware of exceptions to the general correlation noted. The data supporting this observation (*1–4*) are summarized in Table 1. This communication attempts to rationalize these observations on the basis of a simple scheme of reactivity.

TABLE 1

COMPARATIVE DATA OF SEVERAL ARYLSULFATASE ENZYMES TOWARD *p*-NCS AND *p*-NPS

Source of sulfatase	$pH_{max}^{p\text{-NCS}}$	$pH_{max}^{p\text{-NPS}}$	$V_{p\text{-NCS}}/V_{p\text{-NPS}}$	Reference
Ox liver, type A	5.0	5.4	3	(*1*, *2*)
Patella vulgata	5.3	5.6	>1	(*2*)
Ox liver, type B	5.6	5.7	55	(*2*)
Proteus vulgaris	5.7	6.2	>1	(*3*)
Helix pomatia	5.8	5.8	4.3	This work
Aspergillus oryzae	5.9	6.2	0.7	(*1*)
Charonia lampas	6.2	6.1	>1	(*2*)
Aerobacter aerogenes	6.4	7.12	0.7	This work
Proteus rettgeri	6.7	8.3	>1	(*4*)
Ox liver, type C	7.5	8.0	0.5	(*2*)
Alcaligenes metalcaligenes	7.8	8.8	0.14	(*1*)

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Results from measurements of pH-rate profiles for sulfatases of *Helix pomatia* and from *Aerobacter aerogenes* are presented in Figs. 1 and 2, respectively, and represent data typical of the work discussed here. The assay method employed was that previously described (5). The arylsulfatase from *Helix pomatia* was obtained from Boehringer–Mannheim and that from *Aerobacter aerogenes* was from Sigma Chemical Company. The *p*-NPS substrate was purchased from Sigma Chemical Company, while the *p*-NCS substrate was from ICN Pharmaceuticals Company. Other reagents were of analytical grade.

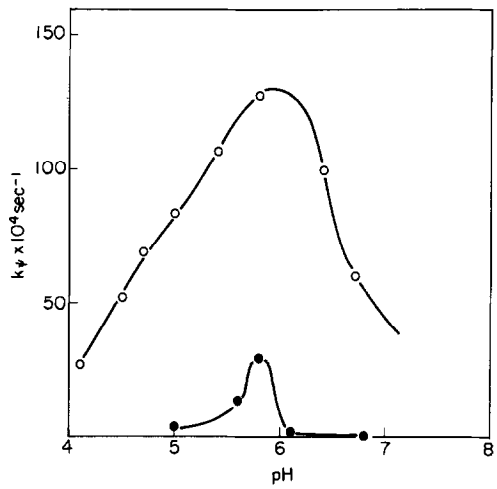
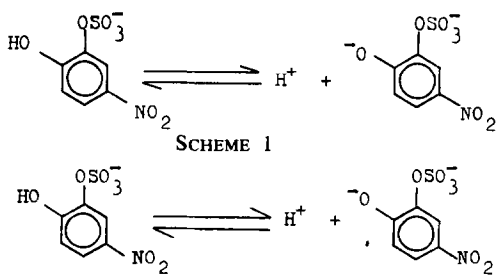


FIG. 1. pH-rate profile for arylsulfatase enzyme from *Helix pomatia* toward *p*-NPS (●) and *p*-NCS (○).

Our results show that for *Helix pomatia* arylsulfatase, the pH_{max} of 5.8 is the same for the two substrates examined (Fig. 1). However, for *Aerobacter aerogenes* arylsulfatase the two pH_{max} differ considerably: $pH_{max}^{p\text{-NCS}} = 6.4$ while $pH_{max}^{p\text{-NPS}} = 7.1$ (Fig. 2). These results could be interpreted as follows: *p*-NPS is a simple substrate which exists in the same ionization form at different pH conditions. However, *p*-NCS has a dissociable phenolic group with a $pK_a = 6.4$ (2) for the equilibrium shown in Scheme 1. Either form, or, as described in Scheme 2, both may be substrate for the arylsulfatases. However, the results of Table 1 and Figs. 1 and 2 strongly suggest that pathway a is overwhelmingly important.



SCHEME 1

a. Enzyme, H_2O → Products

b. Enzyme, H_2O → Products

SCHEME 2

If the undissociated is the preferred form of *p*-NCS as substrate, the general observations i and ii below are explained. (i) The relative activity of any enzyme toward the substrates *p*-NCS and *p*-NPS is a function of pH. $V_{p\text{-NCS}} > V_{p\text{-NPS}}$ at acidic pH while $V_{p\text{-NPS}} > V_{p\text{-NCS}}$ at alkaline pH. (ii) Generally, $pH_{max}^{p\text{-NCS}}$ is more acidic than $pH_{max}^{p\text{-NPS}}$ for each enzyme. This shift is very much larger when pH_{max} occurs in the alkaline side.

All enzymes possess ionizable residues which define their pH-rate profiles toward nonionizable substrates. Arylsulfatases show pH_{max} in the 4–9 range, defined solely by the properties of the enzyme when a simple arysulfate, such as *p*-NPS, is the substrate.

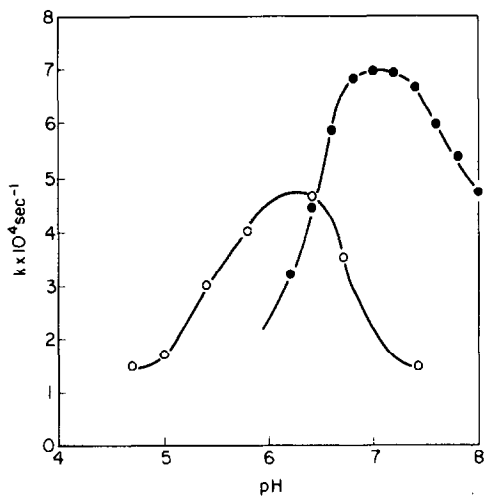


FIG. 2. pH-rate profile for arylsulfatase enzyme from *Aerobacter aerogenes* toward *p*-NPS (●) and *p*-NCS (○).

However, when *p*-NCS acts as substrate, another acid–base variable is introduced, since this compound is ionizable. If it is assumed, as the arguments above suggest, that only the protonated *p*-NCS is reactive towards sulfatases, enzymes with pH-rate profiles in the alkaline side would show very low activity toward *p*-NCS. In addition, this activity would tend to be shifted toward more acidic pH values as a result of a compromise between the enzyme activity and the dissociation curve of *p*-NCS.

Residual reactivity of the dissociated *p*-NCS cannot be excluded with the available data. However, it must be very small, and for the purpose of a qualitative description such as this, pathway b in Scheme 2 may be considered nonexistent.

The situation is illustrated in Fig. 3. Part a shows the case of an arylsulfatase enzyme having a pH-rate profile toward *p*-NPS with ph_{max} ~5. When *p*-NCS is the substrate, practically all of it will be as the phenolic form at pH = 5 ($pK_a^{p\text{-NCS}} = 6.4$). Thus the pH-rate profile towards *p*-NCS would be very similar to that for *p*-NPS.

Figure 3b corresponds to the case in which the enzyme has a pH-rate profile with pH_{max} 6 with *p*-NPS. When *p*-NCS is the substrate, the above pH-rate profile must be combined with the dissociation of the substrate to yield the pH-rate profile for *p*-NCS. As a result, there is a difference in the pH_{max} for each substrate discussed.

Figure 3c shows the case when *p*-NPS has a $pH_{max} \sim 7$, while the combination effect yields a $pH_{max} \sim 6.5$ for *p*-NCS. Also, the reactivity of the enzyme would be higher with *p*-NPS than with *p*-NCS.

Finally, Fig. 3d, shows the case when $pH_{max}^{p\text{-NPS}} \sim 8$. The combination effect would yield $pH_{max}^{p\text{-NCS}} \sim 7$, with a higher reactivity toward *p*-NPS than to *p*-NCS.

The greater reactivity of *p*-NCS than *p*-NPS at acidic pH may derive from a better fit to the steric requirements of the sulfatases by the former substrate. This suggestion is supported by the observation of greater reactivity by *m*- than *p*-NPS (*1*, *5*): the nitro group of *p*-NCS is *meta* with respect to the sulfate function.

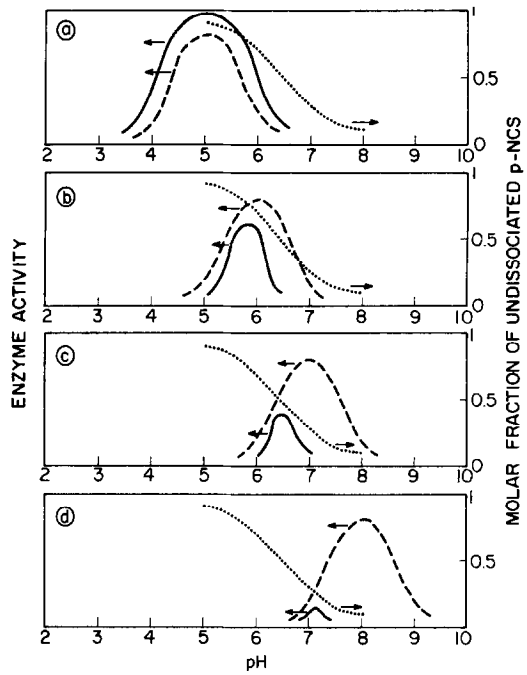


FIG. 3. Schematic representation explaining the relative behavior of different arylsulfatases toward *p*-NPS (---) and *p*-NCS (——) as a function of the phenolic form of *p*-NCS (···) and their $pH_{max}^{p\text{-NPS}}$. (a) Case of arylsulfatase having $pH_{max}^{p\text{-NPS}} \sim 5$. (b) Case of arylsulfatase having $pH_{max}^{p\text{-NPS}} \sim 6$. (c) Case of arylsulfatase having $pH_{max}^{p\text{-NPS}} \sim 7$. (d) Case of arylsulfatase having $pH_{max}^{p\text{-NPS}} \sim 8$.

Examples of each of the above types have been reported [(*1–4*), Table 1]. The situation in 3a fits the data of sulfatases A and B from mammalian origin: $pH_{max} \sim 5$, V_p-NCS > V_p-NPS. Several sulfatases, for example from *Helix pomatia* (Fig. 1), *Aspergillus oryzae* (*1*), *Proteus vulgaris* (*3*), *Patella vulgata* (*2*), and *Charonia lampas* (*2*) gave results compatible to Fig. 3b. Case 3c corresponds to the sulfatase from *Aerobacter aerogenes* (Fig. 2), in which the activity towards *p*-NPS is higher than that to *p*-NCS and also $pH_{max}^{p\text{-NPS}} = 7.1$ while $pH_{max}^{p\text{-NCS}} = 6.4$. Finally, Fig. 3d corresponds to sulfatase C from mammalian sources (*2*) and also to sulfatases such as those from *Alcaligenes Metalcaligenes* and *Proteus rettgeri* (*1*, *2*), which have $pH_{max}^{p\text{-NPS}} \sim 8$ while $pH_{max}^{p\text{-NCS}} \sim 7$ with a small degree of activity for *p*-NCS.

Several explanations could be advanced for the assumed unreactivity of the phenolate form of *p*-NCS. For example, shielding charge effects of ionized groups at the active site could preclude the reaction between the enzyme and the phenolate form of the sulfate. Alternatively, the phenolate oxygen could divert the operation of a H-bonding function in the enzyme which could interact at the sulfate oxygen.

Whatever the correct explanation of this phenomenon, it is evident that *p*-NCS is a complex substrate and that its behavior is linked to its acid–base characteristics. This fact may be useful in designing experiments for the study of the mechanisms of arylsulfatases.

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